

# **Impact of the conjugate vaccine, MenAfriVac, on carriage of serogroup A *Neisseria meningitidis* and disease transmission**

by

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## SUMMARY

*Neisseria meningitidis* (Nm), also referred to as meningococcus, is a human commensal colonising the oropharynx, transmittable by close contact between healthy people. The bacterium can act as an opportunistic pathogen and cause bacterial meningitis and septicaemia. Meningococci are classified into 12 serogroups based on the composition of their polysaccharide (Ps) capsule. Six of these serogroups, serogroups A, B, C, W, X and Y cause meningococcal disease worldwide. The populations most affected by the disease live within the meningitis belt, a sub-Saharan region stretching from Senegal to Ethiopia. Serogroup A Nm (NmA) has been responsible for most of the outbreaks and large epidemics in that region, but serogroups W and X have also caused outbreaks.

Until 2010 Ps vaccines were used to halt ongoing epidemics in the meningitis belt, although they had limited effect in young children and did not provide long-term immunity. Conjugate vaccines, developed by coupling a protein to the Ps antigen to activate the T-helper cells of the immune system, elicit strong and long-lasting immune responses, also in children under 2 year, and mediate immunological memory. Until recently, however, conjugate vaccines were too expensive for African countries. An affordable tetanus toxoid-conjugated NmA vaccine, MenAfriVac, was therefore developed for use in the meningitis belt. Burkina Faso was the first country to vaccinate its population between 1 and 29 years of age in December 2010.

In addition to protect vaccinated persons from disease, conjugate vaccines may prevent colonisation by the bacterium and, consequently, reduce the circulation of pathogens; indirectly protecting the non-vaccinated people. This herd immunity effect is a major advantage, multiplying the public health impact of a vaccination campaign.

Safety and immunogenicity of MenAfriVac was demonstrated in clinical trials but the potential herd immunity effect of vaccination was unknown. The research summarized in this thesis was therefore initiated to determine whether MenAfriVac could provide herd immunity when used for the first time in mass vaccination in Burkina Faso. The specific aims of this work were to: 1) assess meningococcal carriage prevalence before and after mass vaccination with MenAfriVac to study the epidemiology of carriage and to evaluate the impact of vaccination on NmA carriage; 2) analyse the genetic and phenotypic characteristics of carriage isolates in comparison to those of invasive isolates from Burkina Faso; and 3) study the mucosal immune response to the vaccine.

A multicentre repeated cross-sectional study of meningococcal carriage in the 1 - 29-year-old population in Burkina Faso was successfully implemented before and after mass vaccination (Papers I, III and IV). Between January 2009 and November 2011, nine sampling campaigns were conducted simultaneously in three districts; the urban district of Bogodogo and two rural districts of Dandé and Kaya. During each sampling campaign >5000 oropharyngeal swabs were obtained from a representative portion of the population within four weeks, yielding a total of 45,847 samples. Samples were analysed by conventional laboratory methods in Burkina Faso and meningococci were confirmed and genetically characterized in Norway. A laboratory quality control system was implemented to harmonize operational procedures and to document the quality and reliability of the results (Paper II).

Overall baseline meningococcal carriage was 3.98% while NmA prevalence was 0.39%. In 2011, after MenAfriVac mass vaccination, overall meningococcal carriage prevalence was 7.02%, while NmA carriage was completely eliminated ( $P < 0.001$ ); NmA was no longer circulating from 3 weeks up to 13 months after mass vaccination both in the vaccinated and unvaccinated individuals. Carriage of NmY sequence type (ST)-4375 dominated in 2009, while carriage of NmX ST-181 dominated in 2010, before and after vaccination, and in 2011, after vaccination, although its prevalence decreased gradually.

The age distribution of meningococcal carriers in 2009, when NmY dominated, was different from the distribution in 2011, when NmX dominated. Thus, age distribution of meningococcal carriage depended on the epidemiological context and the dominant serogroup. Carriage was lowest in urban Bogodogo compared to the two rural districts. We observed a seasonal variation with higher carriage prevalence during the dry period of the year compared to the rainy season.

A study of invasive isolates recovered from patients in the meningitis belt between 2004 and 2010, before the introduction of MenAfriVac (Paper VI) showed that meningococcal disease had predominantly been caused by NmA ST-7 and ST-2859, both clones belonging to the ST-5 clonal complex. In Burkina Faso, the same genotype of NmA (ST-2859;P1.20.9;F3-1) was found in healthy carriers and in patients throughout the study period, NmX ST-181 emerged in 2010 before MenAfriVac introduction, NmY ST-4375 was sporadically identified, while the virulent NmW ST-11 clone was not circulating after 2004.

Molecular characterization of carriage and disease isolates after vaccine introduction demonstrated that NmW ST-11 had been re-introduced in Burkina Faso after MenAfriVac vaccination as it was first detected among carriers and patients in 2011 (Paper IV). We showed that higher NmX carriage and disease after vaccine introduction was not vaccine-

induced, but due to a clonal wave that started before vaccine introduction. Capsule switch of the virulent ST-2859 genotype as a result of immune evasion was not observed up to 13 months after vaccination.

The epidemiology of carriage and molecular characteristics of the non-pathogenic, closely related species, *Neisseria lactamica* was also assessed before and after vaccine introduction (Paper V). *N. lactamica* carriage prevalence was homogeneous in the three study districts and stable over time, showing that the vaccine introduction did not affect carriage of *N. lactamica*. The epidemiology and molecular characteristics of *N. lactamica* were similar to those in industrialized countries; overall carriage prevalence was 18.2%, with a maximum in 2-year-olds (40.1%), and, in the 18 - 29-year-olds, carriage in women (9.1%) was higher than in men (3.9%).

Saliva samples were obtained from 198 10 - 14 year old children in the capital city of Ouagadougou before and after vaccination. We found a significant increase of salivary IgA and IgG antibodies targeting the serogroup A capsule after vaccination ( $P < 0.001$ ). As salivary antibodies can prevent acquisition of pathogens in the upper respiratory tract, the results were consistent with the elimination of NmA carriage (manuscript in preparation).

As MenAfriVac was administered to children above 1 year of age in December 2010 and no catch-up or routine vaccination was performed after that, the number of unvaccinated children in Burkina Faso continuously increased. These children, however, were indirectly protected from disease at least up to 13 months after mass vaccination because NmA disappeared from circulation (Paper III). Thus, our results are consistent with a herd immunity effect generated by a successful MenAfriVac introduction.





## SOMMAIRE (summary in french)

*Neisseria meningitidis* (Nm), aussi appelé le méningocoque, est un commensal de l'homme qui colonise l'oropharynx et se transmet entre personnes en bonne santé. La bactérie peut agir comme un pathogène opportuniste et causer la méningite et/ou la septicémie. Les méningocoques sont classés en 12 sérogroupes en fonction de la composition du polysaccharide (Ps) de leur capsule. Six de ces sérogroupes, désignés A, B, C, W, X et Y, causent la méningite dans le monde. Les populations les plus touchées par la maladie vivent dans la ceinture de la méningite, une région de l'Afrique sub-saharienne qui s'étend du Sénégal à l'Ethiopie. Le séro groupe A (NmA) a été responsable de la plupart des flambées et des épidémies importantes dans cette région, mais les sérogroupes W et X ont également provoqué des épidémies.

Jusqu'en 2010 les vaccins Ps ont été utilisés dans la ceinture de la méningite pour arrêter les épidémies en cours, malgré leur effet limité chez les jeunes enfants et leur durée de protection limitée. Les vaccins conjugués, développés en couplant une protéine à l'antigène Ps pour activer les lymphocytes T auxiliaires, provoquent de fortes réponses immunitaires de longue durée, également chez les enfants de moins de 2 ans, et permettent de promouvoir une mémoire immunologique. Cependant, les vaccins conjugués étaient jusqu'à récemment trop chers pour les pays d'Afrique. Un vaccin conjugué contre le NmA à prix abordable, MenAfriVac, a donc été développé pour être utilisé dans la ceinture de la méningite. Le Burkina Faso fut le premier pays à vacciner avec MenAfriVac toute sa population entre 1 et 29 ans en Décembre 2010.

En plus de protéger contre la maladie les personnes vaccinées, les vaccins conjugués peuvent empêcher la colonisation de l'oropharynx et, par conséquent, limiter la circulation des agents pathogènes; et ainsi indirectement protéger les personnes non vaccinées. Cet effet d'immunité de groupe est un atout majeur qui multiplie l'impact d'une campagne de vaccination sur la santé publique.

La sûreté et l'immunogénicité de MenAfriVac a été démontrée dans des essais cliniques, mais l'effet potentiel d'immunité de groupe était inconnue. La recherche résumée dans cette thèse a donc été initiée afin de déterminer si MenAfriVac pourrait fournir une immunité collective, lors de sa première utilisation dans une vaccination de masse au Burkina Faso. Les objectifs spécifiques de ce travail étaient les suivants: 1) évaluer la prévalence du portage du méningocoque avant et après la vaccination de masse avec MenAfriVac, étudier l'épidémiologie du portage et évaluer l'impact de la vaccination sur le portage du NmA; 2)

analyser les caractéristiques génétiques et phénotypiques des souches de portage en rapport avec celles des souches invasives du Burkina Faso, et 3) étudier la réponse immunologique salivaire au vaccin.

Une étude multicentrique transversale répétée du portage méningé dans la population de 1 à 29 ans au Burkina Faso a été mise en œuvre avant et après la vaccination de masse (Publications I, III et IV). Entre Janvier 2009 et Novembre 2011, neuf campagnes de prélèvement ont été menées simultanément dans trois districts, le district urbain de Bogodogo et les deux districts ruraux de Dandé et Kaya. Au cours de chaque campagne de prélèvement plus de 5000 échantillons oropharyngés ont été prélevés chez une portion représentative de la population au cours de quatre semaines, ce qui a donné un total de 45 847 échantillons. Les échantillons ont été analysés par des méthodes de laboratoire classiques au Burkina Faso et les méningocoques ont été confirmés et génétiquement caractérisés en Norvège. Un système de contrôle de qualité a été mis en place pour harmoniser les procédures opérationnelles et pour documenter la qualité et la fiabilité des résultats (Publication II).

Le taux de portage pré-vaccinal du méningocoque était de 3,98% alors que le taux de portage du NmA était de 0,39%. En 2011, après la vaccination de masse avec MenAfriVac, le portage du méningocoque était de 7,02%, tandis que le portage du NmA était complètement éliminé ( $P < 0,001$ ); NmA n'était plus en circulation à partir de 3 semaines jusqu'à 13 mois après la vaccination de masse, à la fois chez les individus vaccinés et les non vaccinés. Alors que le portage du NmY séquence type (ST) -4375 dominait en 2009, NmX ST-181 dominait en 2010, avant et après la vaccination, ainsi qu'en 2011, après la vaccination, bien que son taux de portage ait diminué progressivement.

La répartition par âge des personnes porteuses de méningocoques en 2009, lorsque NmY dominait, était différente de la répartition en 2011, lorsque NmX dominait. Ainsi, l'âge des porteurs du méningocoque dépend du contexte épidémiologique et du sérotype dominant. Le portage était plus faible en milieu urbain que dans les districts ruraux. Nous avons observé aussi une variation saisonnière avec un portage plus élevé pendant la période sèche de l'année par rapport à la saison des pluies.

Une étude des souches invasives isolées chez des patients de la ceinture de la méningite entre 2004 et 2010, avant l'introduction de MenAfriVac (Publication VI) a montré que la méningite avait été principalement causée par NmA ST-7 et ST-2859, deux clones appartenant au complexe clonal ST-5. Au Burkina Faso, le même génotype de NmA (ST-2859; P1.20,9;F3-1) a été trouvé chez les porteurs sains et chez les patients tout au long de la période de l'étude, le NmX ST-181 a émergé en 2010, avant l'introduction de MenAfriVac, le

NmY ST-4375 a été identifié de façon sporadique, alors que le clone hypervirulent ST-11 n'a pas été en circulation après 2004.

La caractérisation moléculaire des souches de portage et des souches invasives après l'introduction du vaccin a démontré que NmW ST-11 a été réintroduit au Burkina Faso après la vaccination avec MenAfriVac; ce clone a été retrouvé chez des porteurs et des patients pour la première fois en 2011 (Publication IV). Nous avons montré que l'augmentation du portage et de la maladie causée par NmX après l'introduction du vaccin n'a pas été induite par le vaccin, mais par une vague de ce clone qui avait commencé avant l'introduction du vaccin. Jusqu'à 13 mois après la vaccination, nous n'avons pas observé un changement de capsule du clone ST-2859, ce qui pourrait s'être produit pour éviter la pression immunitaire.

L'épidémiologie du portage et les caractéristiques moléculaires de la bactérie non-pathogénique, *Neisseria lactamica*, ont également été étudiées avant et après l'introduction du vaccin (Publication V). Le taux de portage du *N. lactamica* était similaire dans les trois districts et stable dans le temps, montrant que l'introduction du vaccin n'a pas influencé le portage de *N. lactamica*. Les caractéristiques épidémiologiques et moléculaires de *N. lactamica* étaient semblables à celles documentées dans des pays industrialisés; le taux de portage était de 18,2%, avec un maximum chez les enfants de 2 ans (40,1%). Chez les 18 à 29 ans, le portage chez les femmes (9,1%) était plus élevé que chez les hommes (3,9%).

Des échantillons de salive ont été obtenus de 198 enfants entre 10 et 14 ans dans la capitale Ouagadougou, avant et après la vaccination. Nous avons constaté une augmentation significative des anticorps salivaires IgA et IgG ciblant la capsule du NmA après la vaccination ( $P < 0,001$ ). Comme les anticorps salivaires peuvent empêcher l'acquisition d'agents pathogènes dans les voies respiratoires, les résultats étaient cohérents avec l'élimination du portage du NmA.

MenAfriVac a été administré aux enfants de plus d'1 an en Décembre 2010 et, comme aucun rattrapage ou aucune vaccination de routine n'ont été effectués par la suite, le nombre d'enfants non vaccinés au Burkina Faso a augmenté. Cependant, ces enfants sont indirectement protégés de la maladie, au moins jusqu'à 13 mois après la vaccination de masse, à cause de la disparition du NmA de la circulation (Publication III). Nos résultats sont cohérents avec un effet d'immunité de groupe généré par une introduction réussie de MenAfriVac.

## ABBREVIATIONS

C3b	Complement fragment 3b
CDC	Centers for Disease Control and Prevention (Atlanta, USA)
CI	Confidence Interval
FetA	Ferric enterobactin transport protein A
fHbp	Factor H binding protein
FrpB	Fe-regulated protein B (same protein as FetA)
GAVI	Global Alliance for vaccine Initiative
GGT	Gamma-Glutamyl Transpeptidase
GPS	Geostationary Positioning System
Ig	Immunoglobulin
IgA	Immunoglobulin class A
IgA1	Immunoglobulin A subclass 1 (IgA2: subclass 2)
IgG	Immunoglobulin class G
LPS	Lipopolysaccharide
MLST	Multilocus Sequence Typing
MoH	Ministry of Health (Burkina Faso)
MVP	Meningitis Vaccine Project
NadA	<i>Neisseria</i> adhesin A
NIPH	Norwegian Institute of Public Health (Oslo, Norway)
Nm	<i>Neisseria meningitidis</i> . NmA, NmX, NmY, ... : <i>Neisseria meningitidis</i> serogroup A, X, Y, ...
OR	Odds Ratio
ONPG	<i>o</i> -Nitrophenyl- $\beta$ -D-galactopyranoside
PATH	Program for Appropriate Technology in Health (a US non-profit organization)
PCR	Polymerase Chain Reaction
PDA	Personal Digital Assistant
PorA	Outer membrane protein Porin A
PorB	Outer membrane protein Porin B
Ps	Polysaccharide
QC	Quality Control
sIgA	Secretory IgA
ST	Sequence Type

TT	Tetanus Toxoid
USAID	U.S. Agency for International Development
VR	Variable Region
WHO	World Health Organization



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# **1. LIST OF PUBLICATIONS**

The following publications (Papers I-VI) are included in the thesis. Reprints are found in Chapter 10.

#### **I.**

**Kristiansen PA**, Diomandé F, Wei SC, Ouédraogo R, Sangaré L, Sanou I, Kandolo D, Kaboré P, Clark TA, Ouédraogo AS, Absatou KB, Ouédraogo CD, Hassan-King M, Thomas JD, Hatcher C, Djingarey M, Messonnier N, Préziosi MP, LaForce M, Caugant DA. **Baseline meningococcal carriage in Burkina Faso before the introduction of a meningococcal serogroup A conjugate vaccine.** Clin Vaccine Immunol 2011; 18:435-43.

#### **II.**

**Kristiansen PA**, Ouédraogo AS, Sanou I, Absatou BK, Ouédraogo CD, Sangaré L, Ouédraogo R, Kandolo D, Diomandé F, Kaboré P, Hassan-King M, Thomas JD, Hatcher CP, Andreasson I, Clark TA, Préziosi MP, LaForce M and Caugant DA. **Laboratory quality control in a multicentre meningococcal carriage study in Burkina Faso.** Trans R Soc Trop Med Hyg 2012; 106:289-297.

#### **III.**

**Kristiansen PA**, Diomandé F, Ba Ki A, Sanou I, Ouédraogo AS, Ouédraogo R, Sangaré L, Kandolo D, Aké F, Saga IM, Clark TA, Misegades L, Dolan J, Tiendrebeogo S, Hassan-King M, Djingarey M, Messonnier N, Préziosi MP, LaForce M and Caugant DA. **Impact of the new serogroup A meningococcal conjugate vaccine, MenAfriVac, on carriage and herd immunity.** Clin Infect Dis 2013; 56:354-63

#### **IV.**

**Kristiansen PA**, Diomandé F, Ouédraogo R, Sanou I, Sangaré L, Ouédraogo AS, Ba AK, Kandolo D, Dolan Thomas J, Clark TA, Préziosi M-P, LaForce FM and Caugant DA. **Phenotypic and genotypic characterization of meningococcal carriage and disease isolates in Burkina Faso after mass vaccination with a serogroup A conjugate vaccine.** BMC Infect Dis. To be submitted

## V.

**Kristiansen PA**, Diomandé F, Ouédraogo R, Sanou I, Sangaré L, Ouédraogo AS, Ba AK, Kandolo D, Dolan Thomas J, Clark TA, Préziosi M-P, LaForce FM and Caugant DA.

**Carriage of *Neisseria lactamica* in 1- to 29-year-old people in Burkina Faso:**

**Epidemiology and molecular characterization.** J Clin Microbiol 2012; 50:4020-27.

## VI.

Caugant DA, **Kristiansen PA**, Wang X, Mayer L, Taha M-K, Bonte L, Ouédraogo R, Kandolo D, and Nicolas P. **Molecular Epidemiology of Meningococcal Meningitis in the African Meningitis Belt before Introduction of a Serogroup A Conjugate Vaccine.** PLoS One 2012; 7:e46019.

The candidate is also co-author of the following publication which is closely linked to the theme of this dissertation, although not part of it:

Djingarey M, Barry R, Bonkougou M, Tiendrebeogo S, Sebgo R, Kandolo D, Lingani C, Préziosi M-P, Zuber P, Perea W, Hugonnet S, DelaPienne N, Tevi-Benissan C, Clark T, Mayer L, Novak R, Berlier M, Toboe D, Nshimirimana D, Mihigo R, Aguado T, Diomandé F, **Kristiansen PA**, Caugant DA and LaForce M. **Effectively introducing a new meningococcal A conjugate vaccine in Africa: The Burkina Faso experience.** Vaccine 2012; 30(Suppl):B40-B45.



## **2. INTRODUCTION**

## 2.1. *Neisseria meningitidis*

*Neisseria meningitidis* (Nm), also known as the meningococcus, is the bacterium responsible for meningococcal disease. Nm belongs to the *Neisseria* genus, named after Albert Neisser for his discovery of *Neisseria gonorrhoeae* (the gonococcus) in 1879 [1]. The genus is composed of a large number of commensal species capable of colonizing mucosal surfaces of humans and some animals [2]. They are all characterized as gram-negative, oxidase-positive and catalase-positive cocci [2]. Within this genus the gonococcus and the meningococcus are the only two species frequently associated with disease in humans [3]. Both species are exclusively human commensals, each specialized in their ecological niche; gonococci in the genitals, meningococci in the upper respiratory tract. *Neisseria lactamica*, another species of the same genus [4], frequently colonises the upper respiratory tract without causing disease, although some rare cases have been reported [5-7].

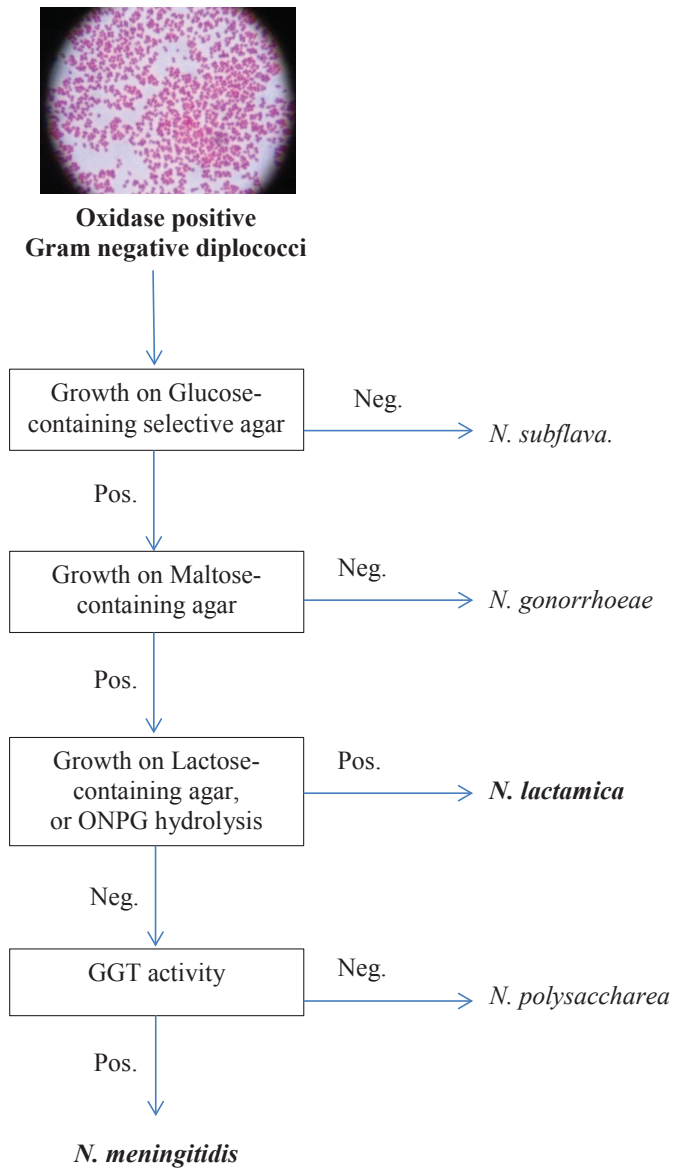
## 2.2. Biochemical properties of *Neisseria* species

The ability to ferment different carbohydrates is used when differentiating the species within the *Neisseria* genus: gonococci are able to ferment glucose only, meningococci can ferment glucose and maltose while *N. lactamica* can ferment lactose in addition to glucose and maltose [3] (Fig. 1). The lactose-fermenting property of an isolate can be detected by the presence of  $\beta$ -galactosidase, an enzyme able to cleave lactose into glucose and galactose, as its reaction with *o*-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) produces a yellow colour (ONPG+; Fig. 2).

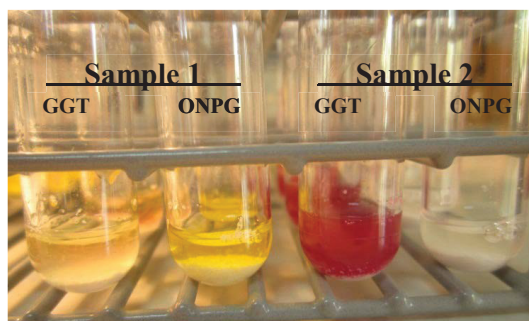
*N. meningitidis* is the only *Neisseria* species possessing the enzyme Gamma-glutamyl aminopeptidase, also called Gamma-glutamyl transpeptidase (GGT), although about 1.2% of meningococcal isolates has been found to lack this enzyme [8, 9]. The possession of this enzyme has been suggested to be advantageous for the proliferation of meningococci in cerebrospinal fluid (CSF) [10]. The presence of GGT can be detected by the enzyme's ability to hydrolyse gamma-glutamyl-beta-naphthylamide and release  $\beta$ -naphthylamine which is detectable by its reaction with an aminopeptidase reagent giving a red colour (GGT+; Fig. 2).

Isolates with an ONPG-negative and GGT-positive test result can be classified as Nm, while ONPG-positive, GGT-negative isolates are *N. lactamica* (Fig. 1 and 2).





**Figure 1. Simple identification scheme for *Neisseria* species**



**Figure 2. Result after enzymatic testing of two bacterial isolates**

Sample 1: GGT- / ONPG+ (*N. lactamica*). Sample 2: GGT+ / ONPG- (*N. meningitidis*).

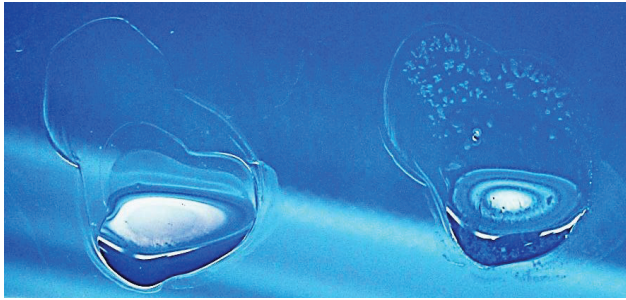
### **2.3. Isolation and identification of *N. meningitidis***

*N. meningitidis* are cultured on agar at 35 - 37°C in CO<sub>2</sub>-rich atmosphere [3]. On agar plates, Nm colonies appear round, smooth, moist and greyish-to-unpigmented. Clinical samples of normally sterile body fluids, such as CSF, are incubated on blood agar plates on which meningococci will likely be seen as a pure culture. For the detection of meningococci from non-sterile sites, such as the throat, their antibiotic resistance is commonly used to eliminate other bacteria from the normal flora [3]. Meningococci grow well on selective agar containing vancomycin (inhibiting Gram-positive bacteria), colistin (inhibiting non-resistant Gram-negative bacteria), nystatin (inhibiting molds and yeasts) and trimetoprim (inhibiting *Proteus* bacteria) [11]. The use of lincomycin instead of vancomycin and amphotericin instead of nystatin is also possible [12].

### **2.4. Classification of *N. meningitidis* into serogroups**

The meningococcus possesses an outer membrane and is protected by a polysaccharide (Ps) capsule. The composition of this capsule is used to further differentiate meningococci into serogroups. Twelve different serogroups have been identified, but only six of them give most

of the cases of invasive disease; serogroups A, B, C, W<sup>1</sup>, X, and Y. The capsule of serogroups B, C, W, X and Y contains sialic acid of different structures, while the serogroup A capsule contains an  $\alpha$ -1,6-linked N-acetyl-D-mannosamine-1-phosphate [13-15]. All these capsules are immunogenic in humans, except for the serogroup B capsule [16, 17]. The determination of serogroup is traditionally done by agglutination tests, such as slide agglutination where serum from rabbits immunized with one type of Ps precipitates in contact with a suspension of bacteria expressing the same Ps capsule [18] (Fig. 3).



**Figure 3. Slide agglutination of *N. meningitidis***

Left: no reaction between the bacterial suspension and antiserum; right: agglutination between the bacteria and the corresponding antiserum

## **2.5. Molecular characterization**

Meningococci can be further classified serologically using the outer membrane proteins PorB (serotype) and PorA (serosubtype), and by using the properties of the endotoxin lipopolysaccharide (LPS; immunotype) [19, 20]. However, the use of molecular methods in the characterization of meningococci has supplemented and in many places taken over the traditional serological identification scheme [20, 21]. Within the bacterial genome, gene segments of interest can be amplified by the polymerase chain reaction (PCR) and analysed by gel electrophoresis or by sequencing. The genes coding for the capsule synthesis can be detected even if the bacteria does not express the capsule [22].

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<sup>1</sup> In a consensus reached at the 18<sup>th</sup> International Pathogenic Neisseria Conference held 9-14 September 2012 in Würzburg, Germany, the designation of *N. meningitidis* serogroup W135 was replaced with *N. meningitidis* serogroup W.

Genes coding for the surface exposed proteins PorA (Porin A) and FetA (Ferric enterobactin transport protein A, also known as Fe-regulated protein B, FrpB) are frequently sequenced to characterize meningococcal strains [23, 24]. PorA and FetA are immunogenic in humans and included as antigens in various vaccines [25-27]. The *porA* gene has two variable regions (VR), VR1 and VR2, and the nucleotide sequences of these regions are used to classify the PorAs into variants [23]. Sequences with  $\geq 80\%$  homology to each other are grouped in VR families and each unique sequence within that family is attributed a variant number. A prototype sequence that defines a new family is designated without variant number. The nomenclature for PorA variants is **P1.VR1 family-VR1 variant number;VR2 family-VR2 variant number**, i.e. P1.5-1,10-1 or P1.20,9. FetA is an iron binding protein the meningococcus can up-regulate, i.e. due to limited availability of iron in the blood [28, 29]. The *fetA* gene has one variable region and different nucleotide sequences are assigned to *fetA* VR families and variants number based on sequence similarity in the same way as described for the variable regions of the *porA* gene [24]. The nomenclature for FetA variants is **FVR family-VR variant number**, i.e. F3-1.

Within the meningococcal genome, housekeeping genes are more conserved than genes coding for surface-exposed components targeted by the immune system as the selection pressure is less important. Seven housekeeping genes have been selected to characterize meningococcal isolates in a multilocus sequence typing (MLST) assay: *abcZ* (putative ABC transporter), *adk* (adenylate kinase), *aroE* (shikimate dehydrogenase), *fumC* (fumarate hydratase), *gdh* (glucose-6-phosphatase dehydrogenase), *pdhC* (pyruvate dehydrogenase subunit), and *pgm* (phosphoglucomutase) [30]. Fragments (alleles) of approximately 400-450 base pairs from each of the seven genes are amplified by PCR and sequenced. The sequence of each allele is compared to the sequences of existing alleles in a web-based reference database [31] and attributed a corresponding allele number if the sequence matches a known sequence, or a new allele number if the sequence is unknown [32]. Together, all seven allele numbers determine a sequence type (ST). Furthermore, STs are grouped into clonal complexes (ST-complex) by their similarity to a central genotype. STs matching a central genotype at four or more loci are assigned to the same ST-complex. This typing method was originally developed for meningococci [30], but MLST is currently used for genotyping of many other species. To date (27.12.2012) there are 10,006 different STs in the *Neisseria* MLST database [31].

## **2.6. Evolution of *Neisseria meningitidis***

The meningococcus is capable of altering its phenotype as a consequence of horizontal gene transfer with other microorganisms [33-36]. DNA uptake sequences, short gene sequences promoting the uptake of external DNA, are mainly situated in the core genome [37]. Although recombination rate for meningococci is high, most of the gene transfer is done with closely related meningococci and is thought to be a DNA repair mechanism of the core genome rather than an evolutionary mechanism [38]. However, new genotypes occasionally emerge and some may take over the ecological niche of their predecessors. New sustainable genotypes can express the same phenotype, although they are more likely to differ in the composition of surface-exposed components such as the capsule (capsule switch) or the PorA or FetA proteins, if it gives them an evolutionary advantage.

## **2.7. Carriage and transmission of *Neisseria meningitidis***

Nm is a strictly human commensal capable of colonising the oropharynx without causing disease [39, 40]. In the carriage state, the bacterium is able to survive and spread in a population by being transmitted between people in close contact either by direct contact (i.e. kissing) or by air-borne droplets (i.e. shouting, sneezing or snoring) [41]. Normally, about 5 - 20% of the population is colonised by meningococci although carriage prevalence can be significantly higher in crowded or contained societies such as universities or military camps, or during outbreaks of meningococcal disease [39, 40, 42-46]. The duration of carriage can vary but carriage of the same clone for 5 - 6 months has been reported [39, 46-49]. For the bacterial population, the carriage state is not only important for its survival and spread, but also for its evolution, as the proximity to other bacteria colonizing the upper respiratory tract may facilitate DNA exchange [35]. Vaginal and anal carriage of Nm has also been reported [50], but oropharyngeal carriage remains the main entrance port for the bacteria.

Meningococcal carriage prevalence in Europe and USA is low during childhood and peaks in adolescence [38, 51]. In sub-Saharan Africa, the age distribution is different, with peak prevalence in younger age groups compared to Europe, but the differences between African countries are significant [44]. In addition to age there are other factors associated with increased risk for meningococcal carriage, such as male sex [43, 52], smoking [53-57], discotheque visits [58], crowding [57, 59] and recent respiratory tract infections [60, 61], while the use of antibiotics is associated with lower risk for carriage [58]. Passive smoking is also a risk factor for meningococcal carriage, but this is likely due to close contact with

smokers with higher carriage rates, rather than exposure to smoke [62]. Although age and sex is often described as a risk factor for carriage, it has been shown that social behaviour of adolescents explains better the higher carriage rates in this age group [63].

Various adhesins, such as the pili and *Neisseria* adhesin A (NadA) have been identified as important for the adhesion and colonization of meningococci to the mucosal epithelium, although the mechanism is not fully understood [39, 64]. The capsule also plays an important role in the colonization process as it protects the bacterium outside the host but once on the epithelial surface, the capsule might sterically hinder adhesion [65, 66]. Thus, the ability to regulate the expression of the capsule is important for the meningococcus. In non-epidemic settings carriage isolates may be capsulated or uncapsulated while during epidemics it is more common to find capsulated carriage isolates [65, 67]. If a meningococcus has successfully colonized an individual, a micro-environment is established around it, favouring the proliferation of the pathogen [68, 69] and transmission to a new host is more likely as the bacterial load is higher i.e. when sneezing.

## **2.8. Meningococcal infection and immunity**

### **2.8.1. The first line of defence**

Although carriage of meningococci does not normally lead to the development of disease, the carriage state is considered as an infection. Mucosal immunity represents the first line of defence against meningococcal infection. In the oral cavity, the adenoid, the palatine tonsil and the lingual tonsil form a defence ring known as the Waldeyer's ring. In these secondary lymphoid tissues, the adaptive immune system can be initiated locally as a response to a mucosal infection and result in locally activated antibody-producing plasma cells [70-72].

Most of the antibodies produced are dimeric IgA [72]. Dimeric IgA can be transported across epithelial cells by binding to secretory component, resulting in secretory IgA (sIgA) antibodies [71]. The function of sIgA is to limit the adherence of pathogens and toxins to the mucosa rather than to activate complement or induce a state of inflammation. The IgA subclass 1 (IgA1) is more abundant on mucosal surfaces than IgA2, although IgA1 is vulnerable to IgA1 proteases produced by Nm and other pathogens [71-73]. Serum-derived antibodies may also contribute in the first line defence against meningococcal infection as they may passively leak onto mucosal surfaces or migrate across epithelia [71, 74].

### **2.8.2. Systemic infection and immune response**

Meningococci are able to cross the mucosal surfaces [65] and invade the blood or the meninges and rapidly cause septicaemia and/or meningitis [20, 75]. The bacteria can rapidly proliferate and release huge amounts of endotoxins, resulting in systemic disease and death within hours. From the bacteria's perspective, non-invasive carriage is the most appropriate strategy for surviving and spreading in a human population as individuals with meningitis or septicaemia are only exceptionally the source of secondary cases [76].

The immune response against meningococcal infection involves both the innate and the adaptive immune system. B-cells may be activated upon recognition of bacterial surface antigens, such as proteins, LPS or repeated carbohydrate structures of the Ps capsule, resulting in antibody production. In secondary lymphoid tissues CD4<sup>+</sup> T-cells can recognize foreign antigen presented by antigen presenting cells such as dendritic cells, macrophages and B-cells inducing the T-cells to proliferate and mature to become various T-helper cells. T-helper cells may interact with B-cells presenting on their surface the same antigen as that specific to the T-cell receptor on the T-helper cells. This interaction leads to the activation, proliferation and differentiation of B-cells into plasma cells and memory B-cells [77].

Memory B-cells are long-lived and rapidly activated for a secondary immune response in case of a later infection with the same antigen. Plasma cells produce antibodies in various secondary lymphoid organs and the antibodies are transported in the blood. Complement-mediated killing, mainly induced by bactericidal antibodies, is the most important defence mechanism and the chance of developing meningococcal meningitis is significantly higher for people with complement deficiencies [78, 79]. Antibodies binding to the meningococcal surface are also recognised by Fc-receptors on macrophages facilitating phagocytosis of meningococci [80].

In the blood the meningococcus has a range of defence mechanisms to avoid the human immune system. They can up-regulate the synthesis of the Ps capsule protecting itself from host killing mechanisms [65, 81]. The meningococcus also possesses a factor H binding protein (fHbp) that recruits human factor H to inactivate complement fragment C3b and by that manner slows down tagging of the bacteria as foreign antigen for subsequent phagocytosis [64, 82].

## **2.9. Symptoms and clinical manifestation of meningococcal disease**

Typical signs for suspecting meningococcal disease are fever, vomiting, stiff neck and haemorrhagic rash. The symptoms, however, are not the same in all age groups [83]. Prompt medical attendance is prescribed as the time to hospital admission is critical for the outcome; patients may die within 12 - 24 h [84].

Systemic meningococcal disease is classified in four main categories: septicaemia, meningitis, septicaemia + meningitis, and mild meningococemia [75]. Septicaemia is the dominant presentation in the Western countries, while meningitis predominates in sub-Saharan Africa [75, 85]. The progression of the disease is associated with the proliferation of meningococci inside the body, as toxic LPS are released upon bacterial death [75, 84]. High LPS levels in blood and low levels in CSF are associated with septicaemia, while low LPS levels in blood and high levels in CSF are associated with meningitis [86].

## **2.10. Medical treatment**

Meningococcal disease is treatable if antibiotics treatment is initiated early enough. If possible, lumbar puncture for the collection of CSF should be performed before treatment as antibiotics will kill bacteria in CSF and make it difficult to diagnose [76]. Parenteral administration of penicillin, ampicillin or amoxicillin is recommended [76, 87, 88]. Ceftriaxone is a good alternative but the cost is higher. Chloramphenicol is a low cost alternative, but its use is not recommended unless there are no other alternative. Most meningococci are susceptible to penicillin, amoxicillin, ceftriaxone, ciprofloxacin, chloramphenicol and rifampin, and resistant to sulphonamides [20, 76, 89]. Resistance to tetracycline has been found in NmA but not in other serogroups [89].

Antibiotic treatment rapidly reduces the contagiousness of patients and eliminates carriage [76, 90]. Thus, in non-epidemic situations, chemoprophylaxis is recommended to prevent secondary cases among close-contacts of patients. However, chemoprophylaxis is not recommended to prevent or control epidemics [76, 91].

With proper treatment and hospitalization, mortality can be reduced to about 10%, from a mortality of 50 - 90% for untreated systemic meningococcal disease [87]. However, many of those surviving meningococcal disease are left with sequelae, such as amputated limbs, loss of hearing or other neurologic damages, leading to significant consequences for the patient and their family, both in term of reduced life quality and in terms of life-long medical treatment and economic burden [92-94].



## **2.11. Global epidemiology of meningococcal disease**

The burden of meningococcal disease and the serogroups responsible for outbreaks vary greatly between regions. The global epidemiology is dynamic in time, affected by the evolution of the bacterium resulting in new clones, by epidemic waves, varying population immunity, vaccination, and long-distance spread facilitated by air travels. Serogroup A outbreaks essentially occur in Africa and Asia, while the serogroups B, C and Y dominate in the U.S. and in Europe, together with serogroup W in Latin America and Africa [95]. Endemic meningococcal disease is caused by various genotypes, while epidemics are often clonal.

The region most affected by meningococcal disease has for a long time been the meningitis belt, a line of sub-Saharan countries stretching from Senegal to Ethiopia [96, 97] (Fig. 4). There, the populations suffer from devastating outbreaks yearly with a peak incidence in the dry seasons and larger epidemics every 5 - 12 years [98, 99]. Outbreaks of NmX and NmW have occurred [100-110], but the major epidemics have been caused by NmA [111, 112]. During epidemics, peak incidence can approach 1000 cases/100,000 [95, 113]. In comparison, the incidence in 2010 was 0.27 cases/100,000 (845 cases) in the U.S [114] and 0.77/100,000 (39 cases) in Norway. The incidence during a serogroup B epidemic in Norway was 7.4/100,000 on average between 1975 and 1985, with a peak incidence of 24/100,000 in Northern Norway in 1975 [115].

Burkina Faso is one of the most affected countries within the meningitis belt. NmA of ST-5, ST-7 and ST-2859 have been successively responsible for most of the outbreaks since the 1990s [89, 111, 116-118]. All these STs belong to the ST-5 clonal complex and all have the PorA variant P1.20,9 and FetA variant F3-1 [89, 116]. In 2002, Burkina Faso was the first country to experience a NmW epidemic [103, 109, 119], which was due to the hypervirulent ST-11 clone [102, 120], probably imported from Saudi Arabia by Hajj pilgrims [121]. In 2006 a major NmX epidemic caused by ST-181 affected the neighbouring country Niger [105]. A few cases of NmX disease were reported in Burkina Faso in 2007, all caused by the same ST-181 clone [89, 122].



**Figure 4. The African meningitis belt** (Reproduced from the MVP web site at <http://meningvax.org>, 2012)

## 2.12. Meningococcal vaccines

Meningococcal disease is preventable by vaccination and as the Ps capsules are targeted by the human immune system during an infection, they can be used as a vaccine component, except for PsB as the sialic acid is identical to that naturally present in human brain tissue [17]. The first vaccines against meningococcal disease were Ps vaccines, used since the 1970's [123]. The disadvantages of Ps vaccines are their lack of immunogenicity in infants, the short duration of protection, the risk of hyporesponsiveness following repeated vaccination, and their questionable ability to prevent transmission [124-127].

For other infections caused by bacteria in the upper respiratory tract, such as *Haemophilus influenzae* and *Streptococcus pneumoniae*, a new vaccine approach was successfully developed and gave birth to conjugate vaccines in the 1980s. By chemically binding capsular Ps to a large protein, B-cells recognizing the Ps antigen can present peptides derived from the protein to CD4<sup>+</sup> T cells. The T-helper cells then activate the B-cells resulting in the differentiation and proliferation of antibody producing plasma cells and memory cells [30]. Conjugate vaccines elicit stronger immune responses that last for a longer time compared to Ps vaccines. In addition, they are immunogenic in infants, in the age group with the highest risk of infection. The first meningococcal conjugate vaccine was available in 1999 (serogroup C) [128], while quadrivalent conjugate vaccines (serogroups A, C, Y, W) were available from 2005 [129, 130]. Although immunological memory is achieved with

conjugate vaccines it is believed that antibody persistence is even more important because meningococcal disease can evolve faster than the body can produce antibodies [131].

Conjugate vaccines developed for use in industrialized countries are not affordable for sub-Saharan African countries. Therefore, the strategy to control meningococcal epidemics in sub-Saharan Africa has been to conduct reactive vaccination with Ps vaccines in response to outbreaks [76]. The choice of reactive and not preventive vaccination is based on the short duration of protection and the lack of immunological memory generated by Ps vaccines. As the pharmaceutical companies have gradually scaled up the production of conjugate vaccines at the detriment of Ps vaccines, the accessibility of Ps vaccines has decreased. A limited emergency stockpile of vaccine is now shared among the sub-Saharan countries, under the management of the World Health Organization (WHO) giving first priority to the most affected countries [132]. Although millions of doses with Ps vaccines have been used for many years to combat meningococcal outbreaks, epidemics keep coming back year after year [122].

### **2.13. Herd immunity**

The primary effect of vaccination is that vaccinated persons obtain immunological protection against later infections. A secondary effect of vaccination is the reduction of circulating pathogens within the population and by that, the indirect protection of unvaccinated people, an effect called herd immunity [133]. The herd immunity effect is important for any vaccination program as it protects (1) the age groups not targeted for vaccination, (2) unvaccinated persons within the targeted age group, and (3) persons not responding properly to the vaccine. If a vaccine can generate herd immunity, mass vaccination might protect the population for a long time without the need for catch-up or routine vaccination. Targeted vaccination of age groups where transmission of the pathogen is highest might be as effective as vaccinating all age groups, and considerably less expensive.

Herd immunity can be assessed by investigating the burden of disease in the non-vaccinated portion of the population. Vaccination of infants in the US with a pneumococcal conjugate vaccine led to a significant reduction in pneumonia caused by the vaccine-prevented serogroups among the elderly population; 90 % of reduction was attributed to herd immunity [134].

Herd immunity can also be assessed indirectly by following the carriage prevalence of vaccine-preventable serogroups in the population after vaccine introduction. Conjugate

vaccines against NmC, *H. influenzae* type b and *S. pneumoniae* have shown to prevent acquisition of the bacterium and by that reduce the circulation of the pathogen [135-142]. A large carriage study conducted in the UK showed that the introduction of a serogroup C meningococcal conjugate vaccine led to a 66% reduction of asymptomatic carriage of serogroup C strains and by this provided herd immunity [136, 137].

## **2.14. The Meningitis Vaccine Project**

As a response to the devastating meningococcal epidemics in the meningitis belt in 1996 and 1997 [113, 122], a public-private partnership between the WHO and Program for Appropriate Technology in Health (PATH) was established in 2000; the Meningitis Project (MVP) [143]. The goal of MVP was to eradicate meningococcal epidemics from the meningitis belt by developing, testing and implementing a safe, immunogenic and affordable NmA conjugate vaccine [144, 145]. Initially funded by the Bill and Melinda Gates foundation, MVP later received funds from various sources, the major contributor being the Global Alliance for Vaccine Initiative (GAVI), USAID, the Dell Foundation and the Centers for Disease Control and Prevention (CDC) ([www.meningvax.org](http://www.meningvax.org)).

This untraditional vaccine development model was a success and MenAfriVac was pre-qualified by WHO in June 2010 allowing its use in the meningitis belt as a single dose to the 1-29-year-olds [144]. The vaccine is manufactured at the Serum Institute of India at an affordable cost for African countries: US\$ 0.4 per dose [143]. A large safety study where 1,200,000 persons received the vaccine took place in September 2010 in Burkina Faso, Mali and Niger [146] and country-wide mass vaccination of the remaining 1-29-year-olds in Burkina Faso was achieved in December 2010 [147]. Mali and Niger introduced the vaccine from the end of 2010 to 2011. A stepwise introduction of the vaccine in the entire meningitis belt was planned, giving first priority to the most affected countries ([www.meningvax.org](http://www.meningvax.org)).

Before the introduction of MenAfriVac in the meningitis belt, clinical trials and safety studies had provided evidence for a safe and effective vaccine [144, 145, 148-150]. The hope was that vaccination would also generate herd immunity as seen with other conjugate vaccines, but this had to be demonstrated as there was no study of the effect of MenAfriVac on carriage.

The introduction of MenAfriVac in the meningitis belt is being monitored through many studies, such as case-based surveillance, case-control study, vaccine coverage assessment and assessment of adverse events [146, 151-154]. The present thesis focusing on

assessing the impact of vaccination on carriage and herd immunity is an important part of the overall assessment of vaccination impact. As the results were obtained in the first country that implemented MenAfriVac, they will be useful for other African countries when considering and planning for vaccine implementation.



### **3. OBJECTIVES AND HYPOTHESIS**

The main objective of this thesis was to explore whether mass vaccination with MenAfriVac, a new NmA conjugate vaccine, could impact the carriage prevalence of serogroup A meningococci, as a reflection of herd immunity.

The main hypothesis was that vaccination with MenAfriVac prevented colonisation by NmA and consequently reduced the prevalence of circulating serogroup A strains in the population.

To answer the main research question, the following studies were initiated:

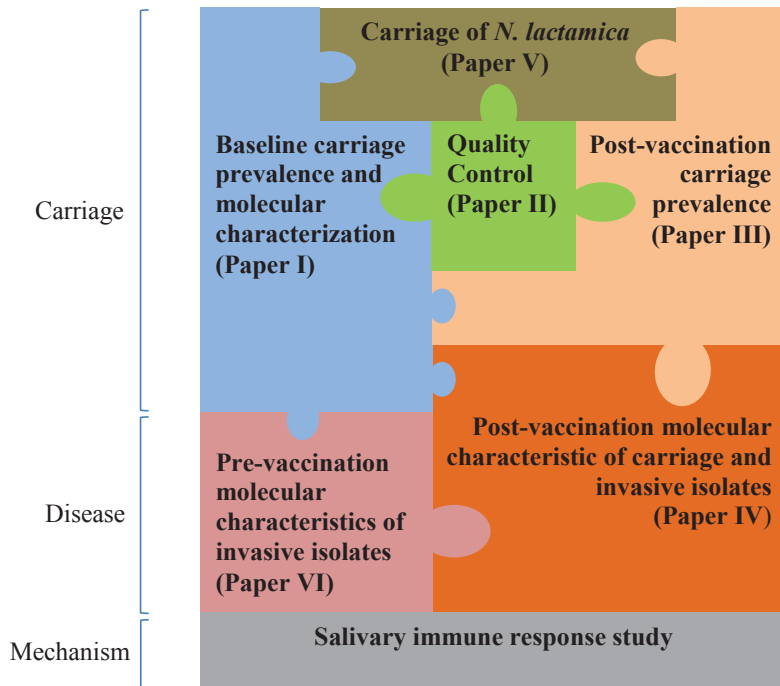
- 1) A multicentre, repeated cross-sectional meningococcal carriage study before and after MenAfriVac vaccination in Burkina Faso
- 2) A study of salivary immune response to MenAfriVac
- 3) A study of the molecular epidemiology of meningococcal disease in the meningitis belt before MenAfriVac vaccination

The studies have different perspectives and the specific results generated by each of them are pieces of evidence that brought together may answer the main research question (Fig. 5).

- The objective of **Paper I** was to determine the baseline carriage prevalence of NmA and other serogroups in 1-29-year-olds in Burkina Faso. We also aimed at describing the epidemiology and molecular characteristics of meningococcal carriage. The initial calculation of sampling size was based on a baseline NmA carriage prevalence of 1% and the assumption that MenAfriVac vaccination would reduce the carriage prevalence by 50%.
- The objectives of **Paper II** were to assess the quality of laboratory diagnostics in the carriage study and to ensure that we obtained reproducible high quality data throughout the study period so that pre- and post- vaccination results would be comparable.
- The objectives of **Paper III** were to determine the post-vaccination carriage prevalence of NmA and other serogroups in the same population as studied before MenAfriVac vaccination, to describe the post-vaccination epidemiology of carriage, and to assess the impact of vaccination on NmA carriage in both vaccinated and unvaccinated populations.



- The objectives of **Paper IV** were to analyse the genotypic and phenotypic characteristics of meningococcal isolates retrieved from the carriage study after MenAfriVac vaccination, in comparison to invasive isolates collected through national surveillance, and to assess the potential immune evasion of invasive genotypes.
- The objectives of **Paper V** were to describe the epidemiology and molecular characteristics of the closely related species *N. lactamica* before and after MenAfriVac vaccination within the population sampled in the carriage study. We assumed that *N. lactamica* carriage prevalence would not be affected by MenAfriVac vaccination, given a low baseline carriage prevalence of NmA.
- The objective of the **salivary immune response study** was to assess the mucosal antibody mediated immunity against serogroup A meningococci after MenAfriVac vaccination. The hypothesis was that the immune response to MenAfriVac led to increased salivary anti-PsA IgG concentrations as a result of leakage from serum.
- The objective of **Paper VI** was to describe the molecular epidemiology of meningococcal disease in the entire meningitis belt in a period of 7 years before the introduction of MenAfriVac.



**Figure 5: Overview of publications and studies, and the connection between them**

## **4. METHODS**

## **4.1. Ethical aspects**

The studies obtained ethical clearance from the Norwegian Regional Committee for Medical Research Ethics, Southern Norway, the Ethical Committee for Health Research in Burkina Faso and the Institutional Review Board at CDC, Atlanta, USA (Papers I, II, III, IV and V).

Data collected in the carriage study were anonymous but, in the salivary immune response study, the name of each participant was registered and we therefore obtained additional approval for the treatment of sensitive data from the Norwegian Data Inspectorate by delegation to the Chief Privacy Officer at NIPH. After inclusion and sampling, the name of each volunteer was kept separate from the laboratory identification number and the final results were non-identifiable to the person's name.

Informed consent was obtained prior to inclusion. The aims of the studies were explained in French and/or tribal language, and an information leaflet signed by the Director General of the Ministry of Health (MoH) was provided.

The analysis of CSF samples from suspected meningitis cases sent to the WHO collaborating centres by national health authorities (Papers IV and VI) did not require additional ethical approvals.

## **4.2. The meningococcal carriage study**

### **4.2.1. Study design**

The study was a multicentre repeated cross-sectional study of carriage prevalence of the 1 - 29-year-olds population in Burkina Faso (Papers I, II, III, IV and V). This specific population was targeted because it was the age-group eligible to receive the vaccine and because implementation of the vaccine was first planned in Burkina Faso.

As carriage prevalence could fluctuate seasonally and vary by region [44, 67, 155], we assessed carriage rates at multiple time points and within one urban and two rural districts (Fig. 6 and 7). A representative proportion of the populations within each district was sampled using a multistage cluster sampling design. The sampling design was established after a pilot study in July 2008 and was chosen as the most rigorous method considering the constraints met in the field, the number of people to include and the limited duration of each sampling campaign.

In the rural districts of Dandé and Kaya, 10 villages were selected by probability proportional to size (Fig. 6). In September 2008, before study start, Socket Mobile 650

Personal Digital Assistants (PDAs) (Socket Mobile, California, USA) equipped with BC-337 compact flash Geostationary Positioning System (GPS) receivers (USGlobalSat, California, USA) were used to register the GPS coordinates of all the compounds in these villages. Before each sampling campaign and for each village, a random selection of GPS coordinates, representing the selected compounds, was uploaded onto PDAs. During each sampling campaign, 8 villages were visited and a team of PDA-operators were able to find the selected compound by GPS navigation.

In the urban district of Bogodogo the approach was different as a city map was available (Fig. 6). All residential blocks within the district border were tagged on a map. A random selection of 16 tags, representing selected blocks, was performed before each campaign. Although selected blocks were somewhat easier to find compared to households in rural districts, we also registered their GPS coordinates in advance and used GPS-enabled PDAs during the sampling campaign to avoid any confusion and to document that the correct block had been visited.



**Figure 6. Geographic distribution of the three districts included in the carriage study.**

The black squares represent the selected villages in the two rural districts.

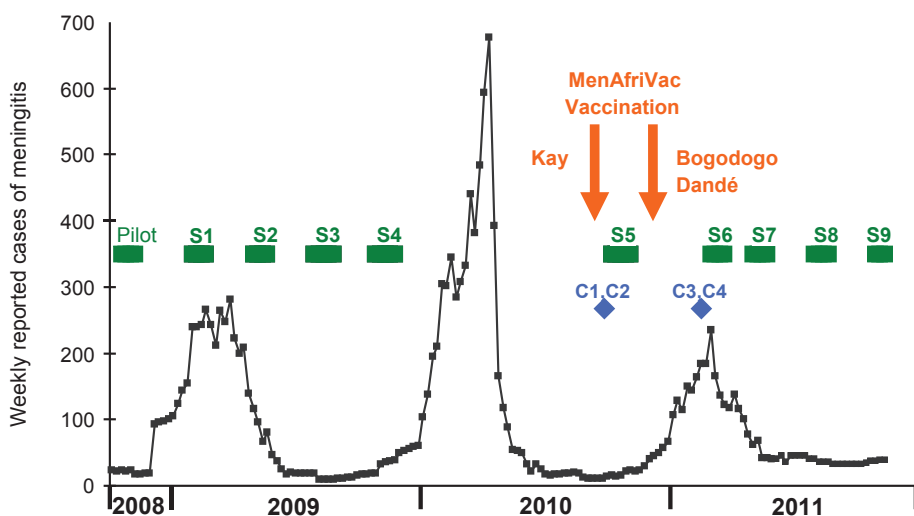
#### **4.2.2. Repeated sampling**

After a large pilot study in October - November 2008, sampling campaigns were organised every three months in 2009 (S1 - S4), in October-November 2010 (S5) and every three months in 2011 (S6 - S9) (Fig. 7). During each of these campaigns >5000 oropharyngeal samples were collected from a representative portion of the populations in the three study districts. Sample collection was done within a 4-week period at a rate of 100 - 110 samples per day, 4 days per week. The vaccine introduction was originally planned for the end of 2009, but due to delays in regulatory authorisations in India, the vaccine was prequalified by WHO only in June 2010 [144]. Because of limited funding, we could not continue with sampling every three months in 2010 as we had done in 2009; the sampling campaigns were halted until October in 2010. By that time, the national health authorities in Burkina Faso had immunized all the 1 - 29-year-olds in the district of Kaya in September 2010, as part of a phased introduction, as recommended by WHO's Global Advisory Committee on Vaccine Safety [146]. The sampling campaign S5 (October - November 2010) was conducted exactly one year after the previous campaign S4 and started three weeks after the mass vaccination in Kaya had ended. Thus, S5 was the first post-vaccination campaign in Kaya and the last pre-vaccination campaign in Bogodogo and Dandé. Mass vaccination of the 1 - 29-year-olds in the remaining districts of Burkina Faso was performed in December 2010; approximately 11.4 million people received the vaccine within 10 days [146].

#### **4.2.3. Enrolment of participants**

The inhabitants of the selected compounds and city blocks were visited at home, and the purpose of the study was explained. Healthy individuals between 1 and 29 years of age, living in the selected houses and present that day, were asked to participate in the study.

Informed consent was registered directly on a PDA by the signature of the head of each household if the compound was composed of multiple households, and of each participant (or parent if < 18 years). A risk-factor questionnaire was administered using a PDA, first at the household level and then for each person. Each participant received a bracelet with a bar-coded Person ID number. This number was registered in the PDA using a Bluetooth connected Socket Mobile 7p barcode scanner (Socket Mobile, California, USA), and linked to the risk-factor questionnaires. The enrolled participants were asked to go to a swabbing station situated at a central location in the village or the city block.



**Figure 7. Timelines for the sampling campaigns S1 - S9 in the carriage study (green) and C1 - C4 in the salivary immune response study (blue), and for MenAfriVac vaccination campaigns (orange), represented with the number of weekly reported cases of meningitis in Burkina Faso (black line) [122].**

#### **4.2.4. Oropharyngeal sampling and identification of meningococci**

Oropharyngeal samples were obtained from enrolled participants by swabbing the posterior pharyngeal wall behind the uvula and one tonsil with a sterile cotton swab (Copan, Italy) (Fig. 8). The swab was immediately plated onto one third of a selective agar plate with modified Thayer Martin VCNT (prepared by WHO/Multi Disease Surveillance Centre, Burkina Faso), containing Columbia agar base (Oxoid, UK), vitox supplement (Oxoid, UK), bovine haemoglobin (BD, Maryland, USA), 3 mg/l vancomycin, 7.5 mg/l colistin, 12.5 U/l nystatin, and 5 mg/l trimethoprim lactate (BD, Maryland, USA). The deposited sample was further streaked out by the use of a sterile loop (NUNC, Denmark) (Fig. 8).

Each plate was labelled with a unique Lab ID number used to identify the sample during the laboratory analysis. To obtain a link between laboratory result and the information of the person swabbed, the barcoded Lab ID and Person ID numbers were both scanned for registration in a PDA.



**Figure 8. Oropharyngeal sampling in the field**

(a) Oropharyngeal swabbing; (b) Plating of sample on selective agar.

In the field, inoculated plates were placed in jars (Remel, Georgia, USA) (Fig. 9). When these were full with a maximum of 42 agar plates, humidified paper and CO<sub>2</sub>-generating pouches (Oxoid, UK) were placed in the jars before sealing them. The jars were then kept at ambient temperature until arrival at the laboratory (within a maximum of 6 h after sampling). The temperature of the jars was monitored and action was taken to avoid temperatures above 37°C.



**Figure 9. Temperature monitoring of inoculated plates in the field**

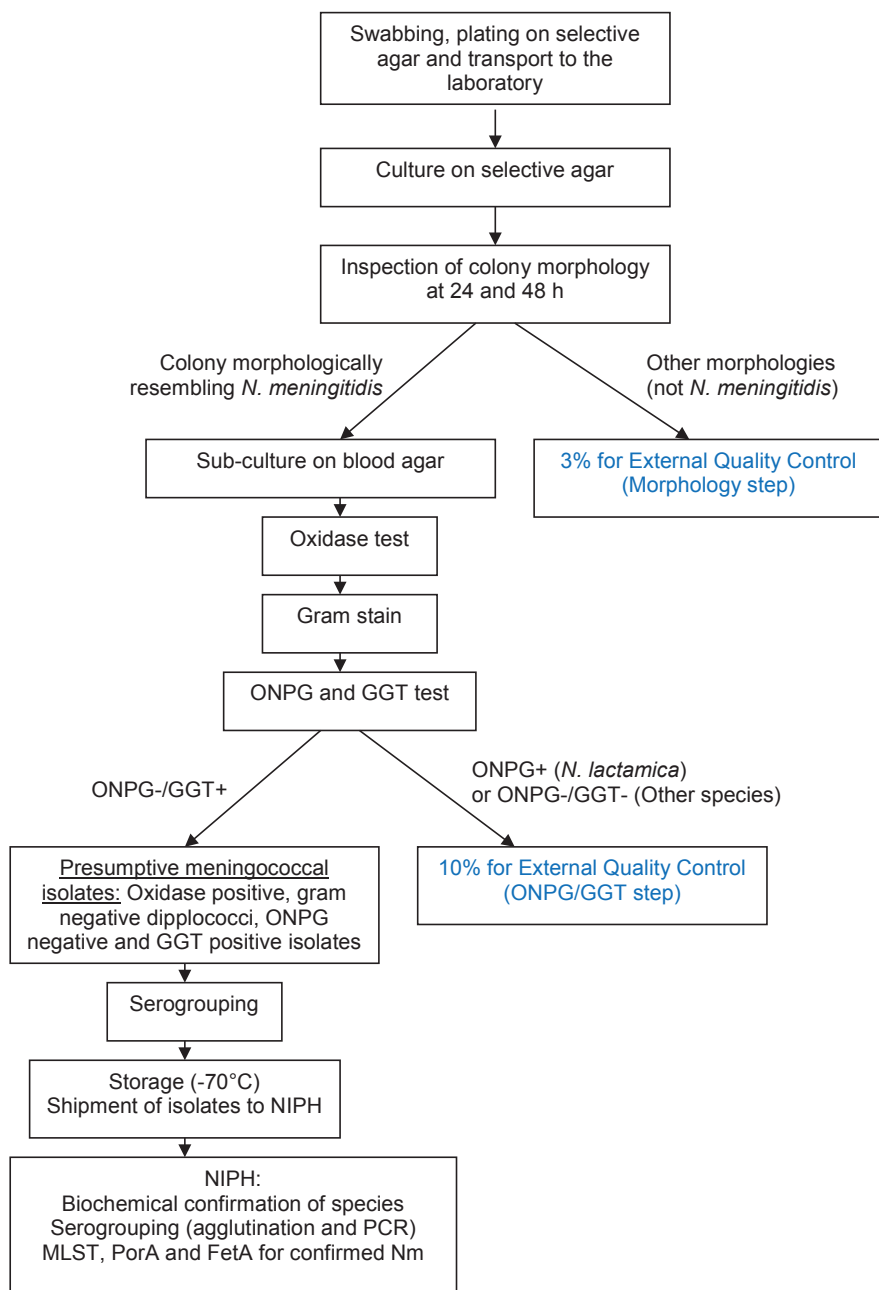


Identification of meningococcal colonies was performed by subculture on Columbia blood agar plates containing 5% sheep blood (Reactivos Para Diagnostico, Spain) at designated hospital microbiology laboratories: the Centre Hospitalier Universitaire Pédiatrique (CHUP) Charles de Gaulle, Ouagadougou for the Bogodogo district; the Centre Hospitalier Universitaire Souro Sanou, Bobo-Dioulasso for the Dandé district; and the Centre Hospitalier Régional de Kaya for the district of Kaya. Standard methods were used to identify meningococci, including Oxidase test (BD, Maryland, USA), Gram staining (BD, Maryland, USA), ONPG and GGT test (Rosco Diagnostics, Denmark), and serogrouping by slide agglutination (Remel, Georgia, USA) (Fig. 10). All ONPG-negative and GGT-positive isolates were considered as presumptive meningococci, suspended in Greaves solution [18] and frozen at -70°C in duplicates. At the end of each sampling campaign, all the isolates were sent to NIPH on dry-ice for confirmation and further molecular characterization.

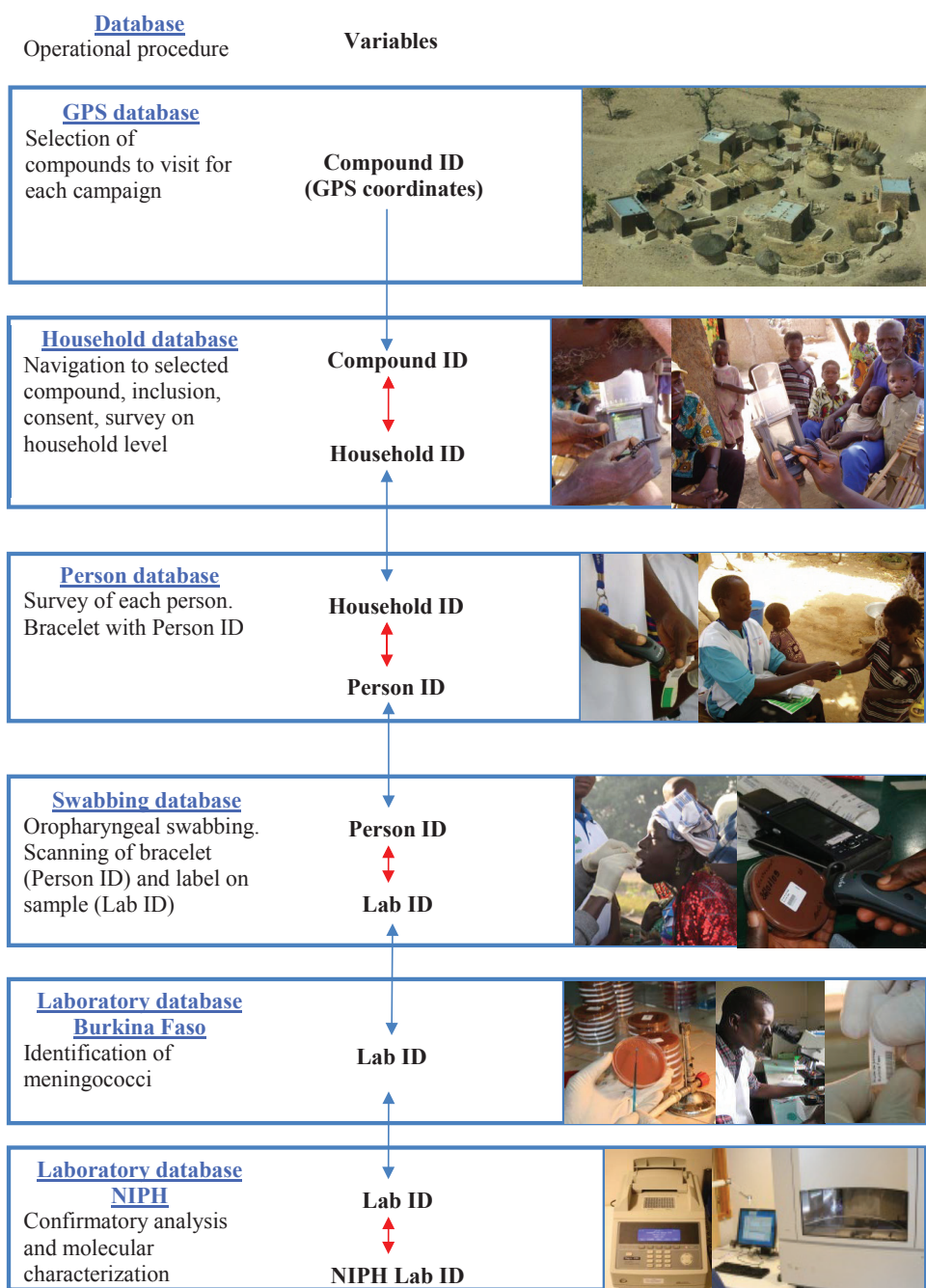
#### **4.2.5. Data management of the carriage study**

The household, person and swabbing databases obtained in the field (Fig. 11) were uploaded and saved onto an external computer on daily basis and backup of the databases was done by supervisors at least every second week. The Lab ID number used as identifier in the national laboratories in Burkina Faso was linked to the NIPH Lab ID used at NIPH (Fig. 11). In all the laboratories, results for each sample were first recorded on a paper register before computerized entry in Access and Excel [156].

Databases from the field, the laboratories in Burkina Faso and the laboratory in Norway were merged into a final database using R version 2.14.1 [157]. Samples were excluded from the analysis if missing or duplicated links between the databases made it impossible to link a laboratory result to the correct person, household or compound.



**Figure 10. Overview of laboratory methods and external quality control**



**Figure 11. Overview of the link between databases (blue arrows), the links between variables within the same database (red arrows) and associated operational procedures.**

### 4.3. Laboratory quality control

One of the key lessons from the implementation and piloting of the carriage study was the importance of harmonization of operational procedures and laboratory quality control (QC). A laboratory QC system was implemented (Paper II) to control the reagents, laboratory equipment and field conditions (internal QC), and to monitor the performance of laboratory identification in Burkina Faso (external QC) (Fig. 10). Internal QC was performed by the laboratories in Burkina Faso while external QC was performed by the NIPH laboratory. External QC consisted in the analysis of a subset of Nm-negative samples to evaluate the proportion of Nm isolates possibly missed during two critical identification steps. All the laboratory analyses performed at NIPH were certified by the Norwegian Accreditation ([www.akkrediter.no/en](http://www.akkrediter.no/en)) and its QC system was in compliance with international standards.

The implemented laboratory QC system was primarily designed to document and, where possible, improve the quality of the results but, in extension, the external QC made it possible to study other isolates than meningococci, such as those of the closely related species *N. lactamica* (Paper V).

### 4.4. Study of *N. lactamica* carriage

To study *N. lactamica* carriage prevalence (Paper V) we used the results from the meningococcal carriage study generated by the laboratories in Burkina Faso. Oxidase-positive, gram-negative diplococci with ONPG-positive test were considered as *N. lactamica* (Fig. 1 and 10). Other variables, such as age and gender were extracted from the same database.

Among the isolates rejected as Nm and sent to NIPH as part of the external QC (Paper II), those confirmed as *N. lactamica* could be further characterized using molecular methods. A representative pool of isolates from all four pre-vaccination sampling campaigns and all the visited villages and city block was randomly selected for molecular characterization.

#### 4.5. Molecular characterization of *Neisseria* species

DNA from each bacterial isolate was prepared by suspending a loop of bacteria growing on agar in 200 µl Tris-EDTA buffer pH 8.0, heating at 95°C for 10 min and centrifuging at 16,000 x g for 5 min. The supernatant was stored at -20 °C until use as DNA template for PCR.

Capsule gene PCR [22] for the identification of the genes coding for synthesis of the Ps capsule was performed on non-serogroupable isolates (Papers I, III, IV and VI) or on culture-negative CSF samples (Paper IV). PorA-PCR [158] for determination of the PorA variant was performed on culture-negative CSF samples (Paper IV).

DNA sequencing of the *porA* and *fetA* genes for the determination of the PorA and FetA variants was performed on all culture-positive samples as described [23, 24]. New gene sequences were submitted to the MLST database (<http://pubmlst.org/neisseria/>) for assignment of new variants.

MLST was performed on *N. meningitidis* isolates (Papers I, IV and VI) and *N. lactamica* isolates (Paper V). Standard procedures were followed (<http://pubmlst.org/neisseria/>) using the primer sets listed in Table 1.

The obtained gene sequences were compared with the sequences accessible on the MLST database and each isolate was assigned to a ST and ST-complex. New allele sequences or new allele number combinations were submitted to the MLST database for assignment of new allele numbers or STs, respectively.

Final laboratory results were entered in Excel [156] and analysed in Excel, R version 2.10.1 [157] or Stata version 11.1 [159]. Phylogenetic analysis was done in MEGA v.5 [160] and START2 [161] and presented as unweighted-pair group method with arithmetic averages (UPGMA) using bootstrap test with 1000 replicates (Paper V).

**Table 1. Primers used for MLST of *N. meningitidis* and *N. lactamica* carriage isolates**

Locus	Primers	Species <sup>1)</sup>	Function <sup>2)</sup>	Ref.
abcZ	abcZ –P1C, abcZ-P2C	M	Amp	[31]
	abcZ-S1A, abcZ-S2	M	Seq	[31]
	abcZ –P1, abcZ –S2	L, M <sup>3)</sup>	Amp, Seq	[30, 162]
adk	adk-P1B, adk-P2B	M	Amp	[31]
	adk-S1A, adk-S2, adk-S1 <sup>3)</sup>	M	Seq	[31]
	C-adk-P1, C-adk-P2	L	Amp, Seq	[162]
aroE	aroE-P1B, aroE-P2B	M	Amp	[31]
	aroE-S1A, aroE-S2, aroE-S1 <sup>3)</sup>	M	Seq	[31]
	aroE-P1, aroE-P2	L	Amp	[30, 162]
	aroE-S1, aroE-S2	L	Seq	[30, 162]
fumC	fumC-P1B, fumC-P2B	M	Amp	[31, 163]
	fumC-S1, fumC-S2	M	Seq	[31, 163]
	fumC-S1, fumC-S2	L, M <sup>3)</sup>	Amp, Seq	[33, 162]
	fumC-P1B, fumC-P2B, fumC-P1, fumC-P2	L	Amp	[33, 163]
gdh	gdh-P1B, gdh-P2B	M	Amp	[31]
	gdh-S3, gdh-S4C, gdh-S1 <sup>3)</sup> , gdh-P2 <sup>3)</sup>	M	Seq	[31]
	L-gdh-P3, L-gdh-P4	L	Amp, Seq	[162]
pdhC	pdhC-P1B, pdhC-P2B	M	Amp	[31]
	pdhC-S1, pdhC-S2	M	Seq	[31]
	pdhC-P1	L	Amp	[30, 162]
	pdhC-P2	L	Amp, Seq	[30, 162]
	pdhC-S1	L	Seq	[30, 162]
pgm	pgm-P1, pgm-P2	M	Amp	[31]
	pgm-S1, Pgm-S2A, pgm-S2 <sup>3)</sup>	M	Seq	[31]
	pgm-S1, pgm-S1	L	Amp, Seq	[162]

<sup>1)</sup> M = *N. meningitidis*, L = *N. lactamica*

<sup>2)</sup> Amp = Amplification, Seq = Sequencing

<sup>3)</sup> Alternative primer set for sequencing

## **4.6. The salivary immune response study**

### **4.6.1. Study design and enrolment**

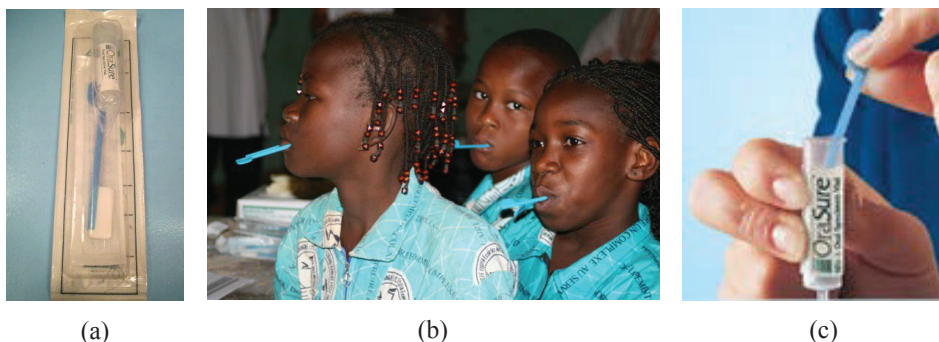
The study was designed as a longitudinal study of a subset of the population targeted for MenAfriVac vaccination, with sampling performed before and after vaccination. A sampling size of 200 individuals was chosen to account for drop outs and the ultimate goal was to obtain at least 150 individuals participating both before and after vaccination. As it was important to sample the same individuals before and after vaccination, and as the temperature and the transport time between sampling and laboratory processing were critical, we conducted the study in the urban district of Bogodogo (Fig. 6), at the mixed school of Bangré, situated nearby the laboratory (CHUP Charles de Gaulle) with students in the age group 10 - 14 years.

Two samplings of the volunteers were performed in October 2010 before vaccine introduction (C1 and C2), and two in February 2011 (C3 and C4), about eight weeks after mass vaccination (Fig. 7). Duplication of the samples before and after vaccination was made to assess the day-to-day variation of antibody concentration.

Prior to the first sampling, the pupils received an information leaflet and a consent form and were asked to return with the consent form signed by a parent, if they agreed to it. At the first sampling, the presence of a signed consent form was checked and registered for each participant, together with their name, age and class. For the subsequent samplings, the name, age and class were used to identify the students. At the first sampling after MenAfriVac vaccination (C3; Fig. 6), the participants were asked whether they had been vaccinated with MenAfriVac and they were encouraged to bring their vaccination card for verification. The participants were asked to abstain from eating or drinking prior to sampling.

### **4.6.2. Collection of saliva samples**

Saliva sampling was performed using the collection device OraSure (OraSure Technologies, Pennsylvania, USA) (Fig. 12a). Two devices were placed between the lower gum and each of the cheeks (Fig. 12b) and left for approximately 2 minutes until the cotton pad was fully saturated. After removal from the mouth, the cotton pad was placed in a tube containing sample buffer (OraSure Technologies, Pennsylvania, USA) and the plastic stick was broken off (Fig. 12c). The samples were kept cool until processing at the laboratory by using temperature monitored polystyrene boxes with frozen ice-packs.



**Figure 12. Sampling technique in the salivary immune response study**

(a) Orasure sampling device; (b) Study participants during sampling; (c) Removal of plastic stick after sampling

#### **4.6.3. Laboratory methods**

Samples were forced out from the sampling device by centrifugation for 5 minutes at 3000 rpm, aliquoted and frozen as soon as possible at  $-70^{\circ}\text{C}$  until shipment to NIPH on dry ice. After arrival in Oslo, the samples were kept frozen at  $-70^{\circ}\text{C}$  until use.

The laboratory analysis performed at NIPH consisted of the quantitation of total IgG and IgA, and anti-PsA specific IgG and IgA. Two ELISA methods to detect salivary total IgG and anti-PsA IgG, and one Bio-Plex method to detect anti-PsA IgA were developed as part of a laboratory project of the Master degree for Lisa Nome [164]. Parameters for the key steps of each developed in-house antibody assay are presented in Table 2. A commercial multiplex bead assay kit, the Bio-Plex Pro Human Isotyping 7-Plex panel, cat no 171-A3001M with 171-304020 (Bio-Rad, USA) was used for total IgA measurements. The multiplex bead assays were performed using Bio-Plex 200 Suspension Bead Array System (Bio-Rad, USA)

Pre- and post-vaccination saliva samples originating from the same child were analysed in the same run. Anti-meningococcal serogroup A/C reference serum pool CDC 1992 (National Institute for Biological Standards and Control (NIBSC), UK) [165] was used as standard curve, allowing the conversion of test output signals to antibody concentrations expressed as ng/ml. Output signals below background signal + 3 standard deviations were considered as not detectable and were, for computational purposes, attributed the value 0.5 in the IgG ELISA and 1.0 in the IgA multiplex assay.

Because of the relatively small sampling size all registrations were done on paper before computerized entry in Excel [156].



**Table 2: Key parameters of the developed in-house antibody assays**

	<b>ELISA (anti-PsA IgG)</b>	<b>ELISA (Total IgG)</b>	<b>Bio-Plex (anti-PsA IgA)</b>
<b>Support</b>	Microtiter plate (Maxisorp, 96 well flat-bottom, NUNC)	Microtiter plate (Maxisorp, 96 well flat-bottom, NUNC)	Diazirine labeled beads (Thermo Scientific 26174; XMAP Reagents, MagPlex Microspheres 029 MC 10029-01)
<b>Coat</b>	5 µg/ml MenA Ps (Serum Institute of India) 5 µg/ml mHSA (NIBSC 04/142) 100 µl / well, 4°C, 2-30 days Blocking solution: 0.5% gelatine (Sigma G2500) 150 µl / well, 37°C, 1 h	2 µg/ml anti-human IgG (Sigma I2136) in 0.02% PBS azid, 100 µl / well, 4°C, 1-30 days	10 µg/ml MenA Ps (Serum Institute of India) UV radiation (380nm): 3x30s 50 µl** / well (Bio-Rad 1711025001)
<b>Sample dilution</b>	2-fold dilutions* from 1:5, in duplicates, 100 µl / well	2-fold dilutions* from 1:100, in duplicates, 100 µl / well	single dilution***, 1:2, in duplicates, 50 µl / well
<b>Standard</b>	NIBSC CDC 1992 (99/706) 2-fold dilutions*, from 100 ng/ml in duplicates, 100 µl / well	Tetagam (Centeon) 2-fold dilutions*, from 150 ng/ml in duplicates, 100 µl / well	NIBSC CDC 1992 (99/706) 2-fold dilutions***, from 675 ng/ml in triplicates, 50 µl / well
<b>Control</b>	Positive and negative control: in-house serum samples, single dilution*, triplicates, 100 µl / well	Positive control: in-house serum Negative control: purified IgM, single dilution*, triplicates, 100µl / well	Positive and negative control: in-house serum samples, single dilution***, triplicates, 50 µl / well
<b>Incubation</b>	4°C, overnight	4°C, overnight	Shaker, 600 rpm, 20°C, 1h
<b>Conjugate</b>	Goat anti-human IgG-ALP (Sigma A3187), 1:20 000 100 µl per well, 37°C, 2h	Goat anti-human IgG-ALP (Sigma A3312), 1:30 000 100 µl per well, 37°C, 2h	PE-labelled Goat anti-human IgA (Southern Biotech 2050-09), 1:2000*** 50 µl per well, 20°C, 1h, 600 rpm

\* ELISA dilution buffer: 0.1% gelatin (Sigma G2500) in Phosphate Buffered Saline (PBS) Tween 0.05%

\*\* Bead buffer: 0.75ml 4M NH<sub>4</sub>SCN + 0.8ml 30% Bovine Serum Ablumine (BSA) + 0.5g skimmed dry milk to 50ml PBS/Tween

\*\*\* Bio-Plex Dilution buffer: PBS Tween 0.05%

#### **4.7. Statistical analysis**

In papers I and II, the Chi-square test was used for the comparison of carriage prevalence in different districts, time points, age groups, etc., and the comparison of the detection rate between swabbers. In papers III and V we applied survey methods to account for correlated data resulting from the cluster sampling design. In addition to chi-square tests, logistic regression was used to calculate odds ratios (OR). Statistical significance was defined as *P* values < 0.05 and as 95% confidence interval not including null. Statistical analysis was done in Stata version 11.1 [159].

In the salivary immune response study, comparison of pre- and post-vaccination antibody concentrations, and day-to-day variation was done by Wilcoxon matched-pairs signed rank test using GraphPad Prism version 5.00 for Windows [166]. Statistical significance was defined as *P* values < 0.05.

#### **4.8. Molecular epidemiology of meningococcal disease in the meningitis belt**

A common strain collection was established between the three existing WHO Collaborating Centres for Reference and Research on Meningococci situated in Oslo, Norway, Paris, France, and Atlanta, USA. Laboratory results of isolates collected from 13 African countries of the meningitis belt between 2004 and 2010 were merged into a common database. Data analysis was done in R version 2.10.1 [157]. This enabled the study of disease-causing strains in the period before MenAfriVac introduction on a molecular level.

## **5. SUMMARY OF RESULTS**

### **5.1. Baseline meningococcal carriage in Burkina Faso before the introduction of a meningococcal serogroup A conjugate vaccine (Paper I)**

The aim of this study was to determine the baseline meningococcal carriage prevalence and serogroup distribution in the 1-29-year-olds in Burkina Faso, before the MenAfriVac vaccine was introduced, to later be able to compare with post-vaccination carriage.

The overall carriage prevalence for meningococci was 3.98%; the highest prevalence was among the 15-19 year-olds for males and among the 10-14-year-olds for females. Serogroup Y dominated (2.28%), followed by serogroups X (0.44%), A (0.39%) and W (0.34%). Carriage prevalence was highest in rural districts and in the dry season, but serogroup distribution also varied by district. A total of 29 STs and 51 PorA/FetA combinations were identified among the 809 isolates. The dominant clone was NmY, ST-4375, P1.5-1,2-2;F5-8, belonging to ST-23 complex (47%). All NmA isolates were ST-2859 of the ST-5 complex, with P1.20,9;F3-1.

In summary we found lower carriage prevalence of serogroup A than expected, but the study formed a solid basis for evaluating the impact of MenAfriVac introduction on NmA carriage and the evolution of carriage of other serogroups.

### **5.2. Laboratory quality control in a multicentre meningococcal carriage study in Burkina Faso (Paper II)**

A major challenge in the carriage study (Paper I) was to harmonize the operational procedures and ensure that the results were reliable. We therefore implemented a laboratory QC system, which is presented in this publication.

Internal QC was performed on media, reagents, laboratory equipment and field conditions. External QC was performed at NIPH on 3% of specimens where no colonies morphologically resembling Nm had been identified in Burkina Faso and on 10% of non-ONPG-/GGT+ isolates (Fig. 10).

The QC system was a critical element: it identified logistic and operational problems in real time and ensured accuracy of the final data. The overall Nm carriage prevalence (3.98%) was probably slightly underestimated and the calculated true prevalence was 4.48%. The components of the presented QC system can easily be implemented in any other laboratory study.

### **5.3. Impact of the serogroup A meningococcal conjugate vaccine, MenAfriVac, on carriage and herd immunity (Paper III)**

The aim of this study was to evaluate the impact of MenAfriVac vaccination on carriage and to address the main research question in the carriage study: did mass vaccination reduce the carriage prevalence of NmA in the population?

In 2010 and 2011 we conducted a repeated cross-sectional carriage study of meningococcal carriage in a representative portion of the 1-29-year-old population in three districts in Burkina Faso in the same way as before vaccine introduction (Papers I and II). One district was vaccinated in September 2010, while the other two were vaccinated in December 2010. Post-vaccination carriage was studied up to 13 months after mass vaccination and compared with pre-vaccination prevalence (Paper I). A total of 45,847 tonsillo-pharyngeal samples were included in the analysis.

In October-November 2010, NmA carriage in the unvaccinated districts was comparable to baseline level but absent in the vaccinated district. NmX dominated in both vaccinated and unvaccinated districts. With four additional sampling campaigns performed every three months in 2011 in the three districts, overall post-vaccination meningococcal carriage prevalence was 6.95%, with NmX dominating but declining for each campaign (8.66 - 1.97%). Overall vaccination coverage in the population sampled was 89.7%, declining over time in the 1-year-old (87.1 - 26.5%), as non-vaccinated infants reached 1 year. NmA carriage was eliminated in both the vaccinated and unvaccinated population from 3 weeks up to 13 months after mass vaccination ( $P = 0.003$ ).

In summary, mass vaccination with MenAfriVac eradicated NmA carriage and conferred herd immunity by preventing NmA colonisation and transmission.

#### **5.4. Phenotypic and genotypic characterization of meningococcal carriage and disease isolates in Burkina Faso after mass vaccination with a serogroup A conjugate vaccine (Paper IV)**

The aim of this study was to document the evolution of circulating meningococcal strains in Burkina Faso up to 13 months after MenAfriVac mass vaccination.

Molecular analysis including MLST and *porA-fetA*-sequencing was conducted on 817 Nm isolates retrieved from the carriage study in 2010 and 2011, and 42 invasive isolates collected through national surveillance in Burkina Faso in the same period.

Seven serogroup A isolates were identified, six in 2010, before vaccination (4 from carriers and 2 from patients), and one in 2011 from an unvaccinated patient; all were assigned to ST-2859. Up to 13 months after vaccination, no NmA carriage isolate and no ST-2859 isolate with another capsule were identified. We found that the meningococcal clones responsible for serogroups A, X and W disease in 2010 and 2011 were the same as the clones of these serogroups dominating among carriage isolates. The increase of NmX carriage and disease prevalence in 2010 was due to the expansion of ST-181, a clone already identified among carriers in 2009 (Paper I). ST-181 represented 48.5 % of all the characterized isolates and 98.1% of all serogroup X isolates. In the most affected district, Kaya, 261 of 266 (98%) characterized NmX isolates were ST-181 and 99.6% of these had PorA variant P1.5-1,10-1.

The hypervirulent ST-11 clone responsible for a serogroup W epidemic in Burkina Faso in 2002 [120] re-emerged in 2011 after vaccine introduction. Comprising 40.6% of the serogroup W carriage isolates, ST-11 was only detected in the districts of Bogodogo and Dandé. Invasive W meningococcal disease incidence increased in 2011 and all the analysed NmW invasive isolates (n = 7) were ST-11.

In light of low pre-vaccination NmA carriage (Paper I), these results complement the findings in Paper III, and provide further evidence that successive clonal waves of ST-181 and ST-11 have contributed to the change in the epidemiology. Thus, the increases of serogroups X and W carriage and disease after vaccination were probably not induced by the vaccine introduction. No capsule switch was observed up to 13 months after vaccination.

## **5.5. Carriage of *Neisseria lactamica* in 1- to 29-year-old people in Burkina Faso: epidemiology and molecular characterization (Paper V)**

The aim of this study was to better understand the epidemiology of *N. lactamica* in Burkina Faso and its relationship to *N. meningitidis*. *N. lactamica* is a true commensal bacterium sharing the same ecological niche as the pathogenic *N. meningitidis* and is believed, to some extent, to protect against meningococcal disease.

From the carriage study (Papers I and III), we were able to identify *N. lactamica* carriage in the 1-29-year-old people living in three districts of Burkina Faso in the period 2009 - 2011, based on common laboratory identification tests.

*N. lactamica* was detected in 18.2% of 45,847 oropharyngeal samples. Carriage prevalence was highest among 2-year-olds (40.1%) and decreased with age. Overall prevalence was higher for males (19.1%) than females (17.5%) (OR, 1.11; 95% CI, 1.04 - 1.18), while among the 18-29-year-olds, carriage prevalence in women (9.1%) was significantly higher than in men (3.9%) (OR, 2.49; 95% CI, 1.94 - 3.19).

Carriage prevalence of *N. lactamica* was remarkably homogeneous in the three districts of Burkina Faso and stable over time, in comparison with carriage of *N. meningitidis* (Papers I and III). There was no significant seasonal variation of *N. lactamica* carriage and no significant change in carriage prevalence after introduction of MenAfriVac.

MLST was performed on a selection of 142 *N. lactamica* isolates from the external QC (Paper II). The genetic diversity was high, as we identified 62 different genotypes, of which 56 were new. The epidemiology of *N. lactamica* carriage and the molecular characteristics of carried isolates were similar to that reported from industrialized countries in contrast to the particularities of *N. meningitidis* carriage and disease epidemiology in Burkina Faso.

### **5.7. Salivary immune response in 10 - 14-year-olds in Burkina Faso after vaccination with a serogroup A meningococcal conjugate vaccine, MenAfriVac (manuscript in preparation)**

A total of 198 participants between 10 and 14 years of age, were included in the analysis, of which 53% were male. All the participants reported to have been vaccinated with MenAfriVac, 66% presented a vaccination card.

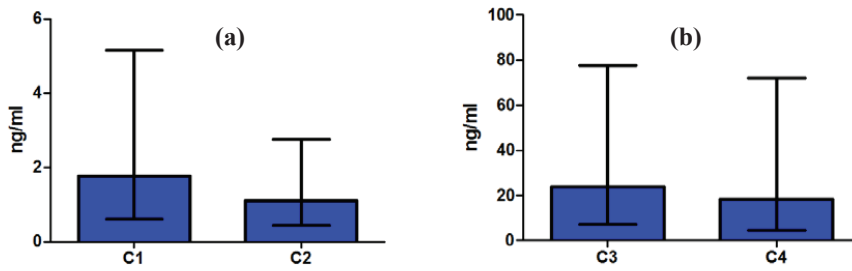
The day-to-day variation of anti-PsA specific IgG, assessed in a random selection of 19 individuals sampled at all four sampling time points was non-significant (Fig. 13). Thus, samples obtained in the campaigns C2 and C4 were used for comparison of pre- and post-vaccination antibody levels, respectively.

While total IgG was somewhat lower after vaccination ( $P = 0.0048$ ), and total IgA concentrations remained stable, anti-PsA IgG and IgA concentrations were significantly higher after vaccination ( $P < 0.001$ ) (Fig. 14). The increase of relative anti-PsA IgG and IgA concentrations, calculated by dividing the titres with total IgG and IgA, respectively, remained significant.

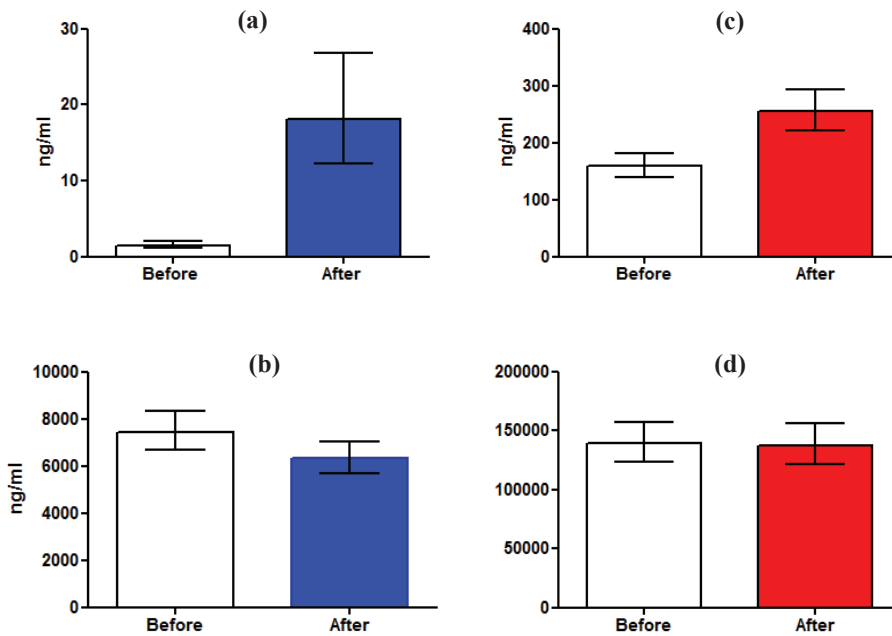
The proportion of individuals with a detectable anti-PsA specific IgG concentration in saliva increased from 22.2% (geometric mean, 1.5 ng/ml) before vaccination to 59.6% (geometric mean, 18.1 ng/ml) after vaccination. Anti-PsA specific IgA was detected in 99% of the participants before vaccination (geometric mean, 158 ng/ml), and 68.7% of the participants had an increase of specific IgA titre after vaccination (geometric mean, 255 ng/ml).

As cross-binding of salivary antibodies may hinder the adherence of meningococci to the epithelial surfaces, the significant increase of anti-PsA IgA and IgG antibodies in saliva after MenAfriVac vaccination contributes to explain the mechanism for protection against serogroup A carriage after MenAfriVac vaccination in Burkina Faso (Paper III).





**Figure 13. Day-to-day variation of specific anti-PsA IgG geometric mean concentrations.** Results from a subset of 19 individuals sampled twice before (a) and twice after (b) MenAfriVac vaccination.  
(a) Sampling C1 and C2, before vaccination; (b) Sampling C3 and C4, after vaccination  
Error bars: 95% CI.



**Figure 14. Salivary antibody geometric mean concentrations before and after MenAfriVac vaccination.**  
(a) Specific anti-PsA IgG; (b) Total IgG; (c) Specific anti-PsA IgA; (d) Total IgA  
Error bars: 95% CI.

## **5.8. Molecular epidemiology of meningococcal meningitis in the African meningitis belt before introduction of a serogroup A conjugate vaccine (Paper VI)**

The aim of this study was to document the characteristics of disease-causing meningococcal strains circulating in the meningitis belt before the introduction of MenAfriVac. A common strain collection from the WHO Collaborating Centres for Reference and Research on Meningococci in Europe and the United States was analysed using standardized methods.

A total of 773 isolates collected between 2004 and 2010 from 13 African countries of the meningitis belt were characterized by MLST, and 487 (62%) of these were also analysed for genetic variation of PorA and FetA proteins. In the strain collection, 578 isolates (74.8%) belonged to the ST-5 clonal complex; 63.7% were ST-2859 and 35.8% were ST-7. These two STs, together with three single-locus variants of ST-7 represented each by a single isolate, characterized NmA strains which have been responsible for most of the epidemics in the period. All these ST-5 complex isolates were remarkably homogeneous in their PorA (P1.9,20) and FetA (F3-1).

Sixty-eight isolates (8.8%) belonged to the ST-11 complex which was mainly characterized by serogroup W, while an additional 38 (4.9%) NmW isolates belonged to the ST-175 clonal complex. Forty-eight (6.2%) NmX isolates from West Africa belonged to the ST-181 complex, while NmX isolates responsible for outbreaks in Kenya and Uganda belonged to an unrelated clone, ST-5403. In addition, various STs were found among 22 NmY isolates.

NmX, ST-181 emerged in Burkina Faso in 2010, before vaccine introduction, causing as many cases as NmA.

In summary there was a slight increase in genetic diversity of the disease-causing strains in the meningitis belt, but serogroup A meningococcal disease caused by the ST-5 complex remained the major epidemic agent.

## **6. DISCUSSION**

## **6.1. Methodical considerations**

### **6.1.1. The meningococcal carriage study**

The choice of direct computerized data entry in the field versus registration on paper was a tremendous success. Training and monitoring of these activities were successful and data safety was handled with several back-up systems. GPS-enabled PDAs have previously been used in similar settings [167]. They provided a robust methodology and enabled a continuous evaluation of the field work and a rapid recapitulation of the laboratory results. Results from laboratory quality control (Paper II) documented that the pre- and post-vaccination results were valid and comparable. Thus, a reduction of post-vaccination NmA carriage was not related to methodological changes or quality issues.

The inclusion of people in the same age group as those targeted for vaccination implied that we would not be able to measure an impact on carriage in older or younger age groups (unvaccinated people). As the whole country was vaccinated there was no unvaccinated control group and it would have been unethical to create such a group for the purpose of this study. Administrative vaccination coverage was estimated at 102.6% based on the number of vaccine doses given and the estimated population size [146]. However, at each carriage study sampling, vaccination status of each participant was recorded and we are able to assess the vaccine coverage in the different age groups. In the last sampling campaign in October 2011, nearly all the 1-year-olds had not received the vaccine as they were too young at time of mass-vaccination campaign. We were therefore able to extract data on the impact of mass-vaccination on carriage in unvaccinated people.

Participants were included if they lived in the selected household and if they were present. It was not unusual that men went to work very early in the morning, especially during the harvest period in the rainy season, while women stayed at home. Although efforts were made to enrol older male participants, children and young females were overrepresented in the sampled population. This selection bias might have contributed to underestimate the overall carriage prevalence, as males were more frequently carriers; but as the bias was similar before and after vaccine introduction, it should not compromise the before- and after-vaccination comparisons.

For the calculation of samples size, we hypothesised a baseline NmA carriage prevalence of 1% and assumed that vaccination would reduce this prevalence by 50%. Thus, a sampling size of 4500 participants was necessary, assuming that a single prevaccination sampling campaign was achievable as vaccination was initially scheduled for the end of 2008.

However, the vaccine introduction was delayed, first to December 2009, then to December 2010. As the baseline NmA carriage prevalence was lower than expected (0.39%; Paper I) and due to potential seasonal variation, we maintained the sampling size for each campaign. Thus, by combining the sampling campaigns, the total sampling size made it possible to demonstrate that the reduction of NmA carriage after vaccination was statistically significant.

When interpreting the results from the *N. lactamica* carriage study (Paper V), an important point was that, although the identification steps for the two species were identical, co-colonisation of Nm and *N. lactamica* was not possible to demonstrate as only one or two bacterial colonies were selected for analysis. Due to the low carriage prevalence of Nm, *N. lactamica* carriage prevalence should be only slightly underestimated.

### **6.1.2. The salivary immune response study**

The sampling size was chosen to assure that at least 150 individuals were included in the analysis. It turned out that the drop-out rate was considerably lower than anticipated: only one individual did not show up for sampling after vaccination due to change of school. We obtained complete data analysis for 198 individuals.

We did not swab the participants in the interest of correlating carriage with salivary antibodies, because the carriage prevalence in this district was so low that it was unlikely to find any NmA carrier. The study would probably have been more significant if we had been able to correlate the results with serum antibody concentrations, but collection of blood samples were not part of the protocol as it was assumed that it might affect acceptance of vaccination.

The choice of sampling procedures was based on previous experience [168]. The choice of introducing multiplex bead assays instead of using only ELISA was based on the higher throughput with the multiplex.

### **6.1.3. Molecular epidemiology of meningococcal disease in the meningitis belt**

The strain collection used to analyse the genotypes causing invasive disease in the meningitis belt before the introduction of MenAfriVac (Paper VI) was not a representative sample of all cases of meningococcal meningitis as the sampling was neither systematic nor proportional to the disease burden. The number of isolates sent to WHO collaborating centres also varied by country, a reflection of differences between the collection systems. Nevertheless, the strain

collection is certainly the most complete collection available from this period. Results from various field studies conducted in the same period were consistent with the results obtained with the strain collection [106, 117, 169].

## **6.2. Epidemiology of meningococcal carriage in Burkina Faso**

### **6.2.1. Age and gender distribution**

In Burkina Faso, asymptomatic carriage of meningococci was found in all studied age groups. If before study start, we had assumed the same age distribution of meningococcal carriage as in Europe, inclusion of teenagers only would probably have been a valid approach. As the age distribution of meningococcal carriage in Burkina Faso was unknown, we included all the age groups that were eligible for vaccination with MenAfriVac and can now conclude that it was the correct approach.

Carriage prevalence was higher among males compared to females both before and after MenAfriVac introduction, in consistency with other studies [52, 63, 67, 170]. The difference in age-specific carriage prevalence between males and females likely reflects differences in social behaviour, as this has been hypothesized to be more important than age or sex [63].

In Burkina Faso, maximum carriage prevalence was found in younger age groups compared to Europe and North America [38, 43, 46, 51, 52, 171, 172]. This difference in age distribution was consistent with studies in other African countries [170, 173-175]. In 2009, the dominating carried serogroup was NmY and the carriage prevalence peaked among the 10-14-year-olds. In 2011, the dominating serogroup was NmX and prevalence peaked in 5-9-year-olds. In both these years, the age group with highest prevalence was given by the dominating serogroup while the other serogroups had very little variation by age. Moreover, the epidemiological context was different in 2011 (mostly NmX and NmW disease), than in 2009 (mostly NmA disease) [122]. Thus, age distribution of meningococcal carriage in Burkina Faso seemed to depend on the epidemiological context and the dominant serogroup.

### **6.2.2. Geographic and seasonal variation**

Previous studies in Africa have reported non-significant seasonal variation of meningococcal carriage despite the significant association between the dry season and epidemic meningitis

[67, 170, 174]. A recent review concluded that there was not sufficient evidence of seasonal variation in carriage prevalence [44]. Waves of clones entering a region have been shown to be more important than seasonal variations [67]. The large sampling size in our study allowed to show seasonal variations in 2009 and 2011 that were statistically significant, with higher carriage prevalence in the dry (epidemic) season. In 2011, however, this observation was blurred by the continuous decline of serogroup X prevalence between October 2010 and November 2011. The reality might be more complex as seasonal variations of carriage might occur in a setting where the circulation of epidemic strains is low, while seasonal variation might be occulted if waves of invasive clones sweep the country [42, 67].

The carriage study was conducted at three locations to account for possible variations within the country. During the whole study period we observed the same pattern with higher prevalence in rural than in urban districts and highest prevalence in Kaya. The difference between rural and urban areas is consistent with previous findings [170]. The district of Kaya is the harshest district in the study, notably drier than the more agricultural district of Dandé, but the living conditions were similar in these rural districts and the selected villages in each district were not very different. The district of Bogodogo has a completely different sociological context with smaller families and higher standard of living, factors that can affect meningococcal carriage. Easy access to medicines, such as antibiotics in the capital might also be a contributing factor.

### **6.3. Relation between meningococcal carriage and disease**

The results from the carriage study (Papers I, III and IV) can be compared to available surveillance data from the same period (Paper VI) [122], without pretending to establish direct causality between carriage and disease.

Serogroup A meningococcal epidemics in the meningitis belt have been caused by isolates assigned to the ST-5 clonal complex since the late 1980s. A shift from ST-5 to ST-7 and later to ST-2859 has been seen in Burkina Faso and ST-2859 has caused outbreaks since 2004 (Paper VI). This clone was also the only NmA clone circulating among healthy carriers in 2009 and 2010 (Papers I and IV). Low NmA carriage prevalence in 2009 and 2010 is consistent with results from other carriage studies in Burkina Faso [155] and other countries in the meningitis belt [170, 174, 176-178] conducted in a non-epidemic period. The epidemic season of 2009 was relatively calm in Burkina Faso with “only” 4,723 reported suspected cases of meningitis; and 30% of laboratory confirmed cases were attributed to NmA. During

epidemics or local outbreaks, however, carriage of NmA can be substantially higher [42, 44, 117].

The emergence of NmX ST-181 in Burkina Faso might be related to an epidemic in Niger in 2006 [105] caused by this clone. NmX ST-181 caused some cases of meningitis in Burkina Faso in 2007 (4% of characterized isolates), but emerged as an important cause of disease in 2010 where it was identified in 7 of the 10 meningococcal isolates sent to the WHO collaborating centre in Oslo (Paper IV). In 2009, NmX ST-181 was identified in 0.44% of healthy carriers in Burkina Faso (Paper I), with a higher prevalence in the north-east district of Kaya, close to Niger (1.05%). The same NmX clone was also present in Benin in 2008 (Paper VI), the neighbour country of both Niger and Burkina Faso (Fig. 4).

NmW isolates assigned to the ST-11 clonal complex have caused meningococcal disease in nearly all the countries of the meningitis belt after the first African epidemic in Burkina Faso in 2002 [103, 179]. In Burkina Faso, this clone was last identified in patients in 2004 (Paper VI) and was not detected among carriers in 2009 and 2010 (Papers I and IV). In 2011 however, NmW ST-11 isolates were found among carriers and patients and an increasing number of patients were identified with this clone in the 2012 epidemic season [122, unpublished data].

Although NmY, dominated by ST-4375, was circulating in Burkina Faso at higher carriage prevalence than NmA (Paper I), this clone did not cause disease during the study period and only sporadically caused meningitis in the years before (Paper VI). The closely related ST-23, a single locus variant of ST-4375 in the *aroE* locus, has been responsible for meningococcal disease in the U.S. and Europe [180-183], but apparently ST-4375 does not possess the same virulence properties. As the outer membrane of these two clones are similar with respect to the PorA and FetA proteins (predominantly P1.5-1,2-2;F5-8) (Papers I and IV) [31], virulence factors may be coded by other regions of the genome than those currently used for strain characterization [184].

Carriage studies give supplementary information about the circulating strains in a population and, to some extent, can be used for epidemic preparedness. However, there is no model able to predict the magnitude of future epidemics based on carriage data. The causal effect of high meningococcal carriage might depend on many variables, such as the invasive potential of the carried isolates, the climate, the population immunity, and the existence of other upper respiratory infections or underlying diseases.



#### **6.4. Vaccine-induced serogroup replacement and capsule switch**

Serogroup replacement, as demonstrated following the use of pneumococcal conjugate vaccines [135, 142], is always a concern when a new vaccine is introduced. Due to low pre-vaccination carriage prevalence of NmA, serogroup replacement was unlikely to occur as a direct result of MenAfriVac vaccination, in the similar way that replacement did not occur in the UK after introduction of a monovalent serogroup C conjugate vaccine [137]. However, a notable increase of NmX cases and a high NmX carriage prevalence was seen in 2011 (Paper III) [122]. NmX was already circulating in the eastern districts of Bogodogo and Kaya in Burkina Faso in 2009, with higher carriage prevalence than NmA (Paper I). NmX was identified among 20% of tested invasive isolates already during the epidemic season of 2010 (before vaccination) [122] and the prevalence of this clone had significantly increased in both vaccinated and unvaccinated districts in October-November 2010 (Paper III). Therefore, we believe that the observed increase of NmX carriage and disease was not vaccine-related. Waning NmX carriage between October 2010 and November 2011 suggested increasing immunity against this serogroup. Most likely the increase of NmX invasive cases in 2011 was due to a wave of NmX, as has been reported elsewhere [67], rather than serogroup replacement due to MenAfriVac vaccination.

NmW has also has been responsible for many cases of meningitis in Burkina Faso after the eradication of NmA disease [122]. Invasive isolates belonged to the hypervirulent ST-11 clone, the same as detected among carriers, but this clone was not detected until 2011, after the mass vaccination campaigns. A particularity of ST-11 in Burkina Faso was that carriage was only detected in Bogodogo and Dandé, possibly because of the dominance of NmX carriage in Kaya. Serogroup replacement would have been a probable explanation for the increase of NmW disease after vaccination if, before vaccine introduction, NmA had occupied a significant ecological niche and the ST-11 clone had circulated in the population. As pre-vaccination carriage prevalence of both NmA and NmW was very low and as the ST-11 was not circulating before vaccination, vaccine-induced serogroup replacement cannot explain the increase of ST-11 disease. A re-introduction of this hypervirulent ST-11 clone 7 years after it last was identified in the country (Paper VI) is a more probable explanation. An increase of NmW disease was observed in Niger in 2010 [106], in Mali in 2011 and by the end of 2012, 14 out of 16 sub-Saharan countries reported laboratory confirmed NmW cases [122].

Another concern when introducing a monovalent vaccine is that virulent isolates expressing the capsule targeted by the vaccine may evade the immune system by taking up genes coding for another capsule [185-187] without losing their virulence [188]. Capsule-switched ST-2859 clones would probably have an evolutionary advantage as ST-2859 has been well adapted to the population in Burkina Faso (Paper VI). However, until 13 months after MenAfriVac vaccination, we did not observe any ST-2859 isolate with a new capsule type.

These results demonstrate the importance of post-vaccination surveillance of both meningococcal disease and carriage, and document the need for an affordable multivalent conjugate vaccine including serogroups A, W and X.

## **6.5. Vaccination coverage**

Through the meningococcal carriage study, we were able to assess the vaccination coverage in the population at multiple timepoints. Compared to the estimated administrative coverage of 102.6% [146] and a national survey documenting 95.9% vaccine coverage [154], we found a substantially lower coverage of 89.7% (Paper III). The discrepancies were probably due to methodological differences: vaccination of people > 29 years and inaccurate population estimates contributed to overestimate the administrative coverage [146], and the national survey was done immediately after vaccination [154].

The decline in vaccine coverage among the 1-year-olds, from 87.1% in October-November 2010 to 26.5% in October-November 2011, was a natural consequence of conducting a single mass vaccination campaign. The number of unvaccinated children reaching 1 year will continue to increase and by the beginning of the 2013 epidemic season, all the children below 3 years and some of the 3-year-olds will not have been vaccinated.

The low vaccine coverage in the 16-29-years-olds at about 85% was consistent with the national survey [154] and with data from Niger where, after mass vaccination with MenAfriVac in 2010, a lower vaccine coverage was found in 15-29-year-olds (87%) compared to 1-14-year-olds (96%) [189]. The adult population represents a potential reservoir for NmA [67], although NmA carriage was higher in younger age groups (Paper I). In future vaccination campaigns these findings should be considered to develop social mobilization strategies targeting this age group.

## **6.6. Impact of MenAfriVac vaccination on herd immunity**

The results presented in this thesis are part of a larger evaluation of MenAfriVac vaccination impact. Other studies [153] indicate that herd immunity was established after a successful mass vaccination of 1-29-year-olds in Burkina Faso with MenAfriVac in September and December 2010.

The carriage study demonstrated that MenAfriVac vaccination eliminated NmA carriage in three districts from 3 weeks up to 13 months (Paper III). With a sampling size of 20,325 before vaccination and 22,093 after, the reduction was statistically significant, in spite of the very low NmA carriage prevalence. Carriage was eliminated in both vaccinated and unvaccinated persons. The reduction of carriage cannot be explained by natural fluctuations as NmA carriage prevalence in October-November 2010 was comparable to the 2009 prevalence for unvaccinated districts, while it had already disappeared in the district vaccinated in September 2010.

In relation to the vaccine introduction, the national surveillance system was improved [146, 153, 190] and, in theory, all suspected cases of meningitis were to be laboratory confirmed. In 2011, a total of 3875 suspect cases were reported and as many as 3125 CSF samples were analysed: only four cases of NmA disease were registered [122, 153], three were migrants from neighbouring countries and one was from Burkina Faso; none of them had been vaccinated. In 2012 none of the laboratory-confirmed cases were infected by NmA. Thus, post-vaccination surveillance clearly demonstrated that serogroup A disease was prevented in Burkina Faso in the two epidemic seasons following MenAfriVac vaccination. Similar results were seen in Mali and Niger after vaccine introduction in 2011 [122].

A consequence of herd immunity is the protection of unvaccinated people. We have shown that NmA carriage was eradicated also in the non-vaccinated population. The number of children too young to have been vaccinated in 2010 increased. The disappearance of NmA disease among these children clearly reflects the beneficial effect of herd immunity. Further studies will be needed to evaluate how long the herd immunity lasts.

## **6.7. Stability of *N. lactamica* population**

The possibility that vaccination might derange the natural balance of the bacterial flora in the oral cavity has been raised, especially for *N. lactamica* as carriage of this bacterium is believed, to some extent, to protect against meningococcal infection by cross-reactive

immunity [191] and as meningococcal vaccines based on *N. lactamica* have been considered [7, 191-194]. However, MenAfriVac vaccination was not expected to have any direct effect on *N. lactamica* carriage and with low pre-vaccination Nm carriage, a reduction of Nm carriage was not expected to leave a large enough niche to disturb this balance. These assumptions were confirmed by remarkable stable *N. lactamica* carriage prevalence at 18.2%; carriage of *N. lactamica* was homogeneous in the three study districts and stable over time, unaffected by changing epidemiology of Nm carriage and disease, or by MenAfriVac vaccination (Paper V). Even with Nm carriage prevalence as high as 25% (Kaya, campaign S5), *N. lactamica* carriage was practically unchanged, at 18.4% (Paper III and V). The estimated carriage prevalence was consistent with that reported elsewhere [43, 172, 174, 195-199].

Characteristics of *N. lactamica* carriage in Burkina Faso, such as age and gender distribution were also stable in time and very similar to what has been documented in other countries (Paper V) [162, 199, 200], despite the particularities of Nm epidemiology in Burkina Faso (Papers I, III, IV and VI). Although the genetic diversity was high, similarities with isolates from the UK [162] were documented (Paper V).

The presented data showed that the epidemiology of *N. lactamica* is uniform in time and space, and independent of Nm epidemiology. FetA variants common to Nm and *N. lactamica* have been identified [201] and further characterization of the *N. lactamica* strain population in Burkina Faso by *fetA* sequencing is planned.

## **6.8. Discovery of a new species?**

One of the *N. lactamica* isolates assigned to a new ST (ST-9100) was genetically very distant from other Nm and *N. lactamica* isolates from Burkina Faso. By comparing its genotype with the genotypes of other *Neisseria* isolates available on the MLST website, it was found to cluster with a group of five other STs registered as *N. lactamica*, the closest being ST-1528 (Paper V). The isolate corresponded phenotypically to *N. lactamica* when using standard enzymatic tests (ONPG+ / GGT-), but in a sugar fermentation test using agar plates, the isolate came out as non-fermenter of either maltose, glucose or lactose. 16S rRNA sequencing revealed only 95% similarity to an uncultured *Neisseria* species clone. There is a possibility that this isolate represents a new *Neisseria* species, but further research is required; whole genome sequencing analysis is ongoing to further characterize this potential new *Neisseria* species.

## **6.9. Salivary immune response to vaccination**

The salivary immune response study, demonstrating that vaccination led to a significant increase of anti-PsA IgG and IgA antibodies in saliva, 8 weeks after vaccination (Fig. 14), is consistent with studies on other Ps based vaccines against pathogenic pharyngeal bacteria [202-208].

Although IgG can be produced locally after stimulation of the mucosal immune system, there is strong evidence that increased salivary IgG after vaccination is serum-derived [203, 206, 208]. Nevertheless, without serum samples we were unable to demonstrate that the increase of anti-PsA IgG after MenAfriVac immunization was serum derived.

The origin of salivary IgA is not investigated in our study as we did not quantify sIgA or serum IgA. Salivary IgA can be produced locally as an immune response to upper respiratory tract infections [71], mucosal vaccination [71] or systemic vaccination [203, 206, 208], or it may be serum derived [71]: the vaccine antigen might be transported from the injection site to various lymphoid tissues such as the adenoids and tonsils, where it triggers the mucosal immune system [202]. The increased post-vaccination anti-PsA IgA concentration in our study could be originating from both local production and from serum.

As 97% of the participants had detectable and high anti-PsA specific IgA before vaccination, while only 22% of them had detectable but low anti-PsA IgG concentration, it seems likely that most of the children had developed a mucosal immune response against an antigen similar to PsA.

As cross-binding of salivary antibodies may hinder the adherence of meningococci to the epithelial surfaces [71, 72], the significant increase of anti-PsA IgA and IgG antibodies in saliva after MenAfriVac vaccination contributes to explain the mechanism behind the documented reduction of NmA carriage (Paper III). Results from the salivary immune response study will be published in the near future [209].

## **6.10. Capacity building**

Laboratory analyses in Burkina Faso are not necessarily very different from those done in Norway, but the conditions are. One of the added values of this project was that national laboratories were supplied with standard laboratory equipment useful for many types of analysis, such as incubators, refrigerators, bio-freezers, safety cabinets, centrifuges, pipettes, etc. The infrastructure of each laboratory was also improved by the purchase of air-

conditioners and maintenance of existing autoclaves and bio-freezers. At the termination of the project, all laboratory supplies (equipment, reagents and consumables) were donated to the laboratories.

The WHO/Multi Disease Surveillance Centre laboratory that produced the selective agar plates for the study was also upgraded with equipment, such as refrigerators, autoclaves, dispensers and media preparation equipment. Its role in supplying media for surveillance purposes in the whole African region was reinforced and the laboratory gained experience in the manufacture and dispatching of large quantities of media as it produced about 6000 agar plates for each campaign.

The implementation of a laboratory quality control system was a success; it provided a system common to all the laboratories that may be applicable to many activities in the future. Some of the laboratories continued with such quality controls as part of their routine activities.

As a support to the participants in the salivary immune response study, the school hosting the study received educational supplies, sport equipment and medical equipment for the school pharmacy. Such support was based on the needs expressed by the school administration and enabled all the students to benefit from the participation in the study.

The training of young technicians was probably the most important aspect of the capacity building. We organized several training sessions and the laboratories became expert in the detection and identification of meningococci. Some technicians also gained experience in saliva sampling. At the laboratory they became used to receiving, storing and controlling laboratory supplies, something normally only a dedicated person was allowed to do. The operators handling PDAs in the field became familiar to PDA technology and were able to solve most occurring problems by themselves. All the participants, including the partners have through this project gained valuable experience in the planning and implementation of a large field study. The skills of the technicians and supervisors participating in this project are an asset for the country and also for the scientific community.

## **7. CONCLUSIONS**

The current carriage study is to our knowledge the largest ever conducted. Many lessons have been learned during the carriage study and the salivary immune response study and the protocols and methods can be further developed and possibly simplified for the future. In conclusion, the main results obtained through this thesis can be summarized as follows:

### **7.1. Epidemiology of meningococcal carriage and disease before vaccine introduction**

Pre-vaccination meningococcal carriage prevalence was low, NmY dominated while NmA was carried by 0.39% of the population and all the NmA strains were identical: ST-2859 (Paper I). There was geographic and seasonal variation of carriage with higher prevalence in the dry season and in rural area. The implementation of a laboratory quality control system ensured a continuous focus on quality and documented the validity of the results (Paper II).

Surveillance of bacterial meningitis between 2004 and 2010, before introduction of MenAfriVac showed that NmA ST-2859 caused most of the outbreaks in Burkina Faso but that disease caused by NmX ST-181 increased in 2010, before the vaccine was introduced (Paper VI). The hypervirulent NmW ST-11 clone was last detected among patients in 2004 and was not identified among carriers in 2009 or 2010 (Papers I, III and VI).

There was no geographic or seasonal variation of *N. lactamica* carriage and its prevalence remaining stable in time at an average of 18.2% (Paper V). The epidemiology and genetic diversity of *N. lactamica* carriage in Burkina Faso was similar to that observed in industrialized countries. We identified a carriage isolate, phenotypically identical to *N. lactamica* but genetically distant from both *N. lactamica* and Nm, with a new genotype (ST-9100) that might represent a new *Neisseria* species.

### **7.2. Impact of MenAfriVac vaccination**

Carriage of NmA was eliminated up to 13 months after the introduction of MenAfriVac in Burkina Faso, both in vaccinated and unvaccinated individuals (Paper III). MenAfriVac vaccination induced a significant increase of salivary anti-PsA IgA and IgG antibodies, providing a mechanistic explanation consistent with the decrease of NmA carriage (Salivary immune response study). With a dramatic reduction of meningococcal disease caused by NmA, the results presented here are coherent with a vaccine-induced herd immunity effect.



The ecological niche left by NmA did not affect the carriage prevalence of *N. lactamica* and was not large enough to explain the changes in carriage and disease epidemiology (Paper V). The increase of carriage and disease of NmX after vaccine introduction was probably not vaccine-related, but due to a wave of the ST-181 NmX genotype starting before vaccine introduction (Paper IV). The increase of NmW disease in 2011 was caused by a reintroduction of NmW ST-11.

No capsule switch was observed among the ST-2859 isolates up to 13 months after vaccination (Paper IV).



## **8. FUTURE PERSPECTIVES**

### **8.1. Implications for future vaccination strategies**

After an initial mass vaccination there are several options to consider. If an ongoing clinical study (PsA-TT-007) is satisfactory, MenAfriVac could be included in the routine vaccination schedule and given from the age of 9 months. The vaccine may also be used in catch-up campaigns at certain time intervals immunising all non-vaccinated children above a minimum age. The need for and the timing of a booster dose must also be explored. Prolonged surveillance of meningococcal carriage in addition to disease surveillance and serological studies would help understand the long-term effect of vaccination on carriage and to identify appropriate vaccination strategies.

Our results also highlight the need for making vaccines against serogroups X and W available for African countries. To date, there are NmX vaccines, plain Ps or conjugate.

### **8.2. Dynamic of clearance and possible therapeutic use of vaccines**

The rapid decline in NmA carriage after vaccination poses new questions: How rapidly does carriage decline? Does the vaccine prevent from acquisition or does it also clear ongoing carriage? These questions are relevant when considering the possible use of conjugate vaccines as therapeutic vaccines aiming at hindering the spread of the germ, in the same way as antibiotics can be used as prophylaxis for close contacts of a patient.

### **8.3. Salivary antibodies as correlates of protection**

The salivary immune response study provides the first elements of mucosal immunity data after MenAfriVac vaccination. Further method development and inclusion of secretory component, IgA and IgG subclasses and serum antibodies as targets in future studies would contribute to a better understanding of the salivary immune response to conjugate vaccines. There are still many unknown elements. For how long does the salivary IgA and IgG persist? Are the mucosal anti-Ps antibodies serum derived? How will the salivary immunity respond to a vaccine booster dose? By which mechanisms do the antibodies prevent acquisition and possibly clear carriage? In view of future vaccine development and population studies, finding correlates for protection against meningococcal carriage and/or infection by measuring salivary antibodies would be an enormous asset.

#### **8.4. Continued surveillance of meningococcal carriage and disease**

The study has documented the impact of MenAfriVac on carriage up to 13 months after vaccination. We were able to organize an additional sampling campaign in October-November 2012, documenting the carriage situation 2 years after vaccination. Preliminary results showed that NmA carriage was nearly inexistent but that NmW carriage had become predominant at alarming levels. This information was immediately shared with the MoH enabling a better preparedness for the coming epidemic season of 2013.

As Burkina Faso was the first country to fully implement MenAfriVac, emphasis should be made to continue the carriage study with at least one sampling campaign per year to document the carriage epidemiology for another 5 or 10 years or, at least, until carriage of NmA returns to the same levels as before vaccination.

#### **8.5. Transformation of unique data and strain collection into knowledge**

We have assembled a unique dataset that can be further analysed. The epidemiology of carriage can be further studied and multivariate risk factor analysis will be published in the near future. With GPS-coordinates of each household and for each sample taken, it is possible to look for households with carriers at multiple time points to study of the dynamic of carriage. Another possibility is to link the results to a database containing climatic data for Burkina Faso from the same period. It could generate new knowledge about the climatic factors affecting meningococcal carriage.

The unique strain collection arising from the project can also be subject to many future studies, such as studies of the differences between invasive isolates and carriage isolates, the search for virulence genes or studies looking at vaccine antigens in meningococci circulating in this population.



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## **10.PUBLICATIONS**























**Phenotypic and genotypic characterization of meningococcal carriage and disease isolates in Burkina Faso after mass vaccination with a serogroup A conjugate vaccine**

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# **Abstract**

## **Background**

The conjugate vaccine against serogroup A *Neisseria meningitidis* (NmA), MenAfriVac, was first introduced in mass vaccination campaigns of 1-29-year-olds in Burkina Faso in 2010. The aim of this study was to genetically characterize meningococcal isolates circulating in Burkina Faso before and up to 13 months after MenAfriVac mass vaccination.

## **Methods**

Carriage isolates were collected in a repeated cross-sectional carriage study of the 1-29-year-olds in three districts of Burkina Faso in 2010 and 2011, before and up to 13 months after mass vaccination. Invasive isolates were collected through the national surveillance in Burkina Faso in the same period. The isolates were characterized by serogroup, multilocus sequence typing and *porA-fetA* sequencing.

## **Results**

A total of 817 carriage isolates and 42 invasive isolates were genotyped. Seven serogroup A isolates were identified, six in 2010, before vaccination (4 from carriers and 2 from patients), and one in 2011 from an unvaccinated patient; all were assigned to sequence type (ST)-2859. No NmA carriage isolate and no ST-2859 isolate with another capsule were identified after vaccination. Serogroup X (NmX) carriage and disease prevalences were high due to the expansion of ST-181, representing 48.5 % of all the characterized carriage isolates and 98.1% of the NmX isolates in the district of Kaya, where NmX carriage was highest. The hypervirulent serogroup W (NmW) ST-11 clone was not observed before vaccination, but appeared during the epidemic season of 2011, representing 40.6 % of the NmW carriage

isolates. Carriage of ST-11 was first detected in the western study site of Dandé and spread to the central study site of Bogodogo, but did not reach the eastern site of Kaya.

## **Conclusions**

The increase of NmX carriage and disease in 2010 and 2011 started before vaccine introduction and was caused by ST-181 that circulated in Burkina Faso already in 2009. The NmW ST-11 clone responsible for higher incidence of meningococcal disease in 2011 and 2012 was re-introduced in Burkina Faso in 2011. Thus, the increases of NmX and NmW prevalence after vaccination were probably not induced by the vaccine introduction. No capsule switch was observed up to 13 months after vaccination.

## **Keywords**

*Neisseria meningitidis*, carriage, meningitis, Burkina Faso, conjugate vaccine, MLST, meningitis belt

## Background

In the African meningitis belt, a sub-Saharan region stretching from Senegal to Ethiopia [1,2], the populations are affected by annual outbreaks of bacterial meningitis caused by *Neisseria meningitidis* during the dry season. Devastating epidemics with peak incidence approaching 1000 cases/100,000 [3] occur every 8-12 years. Most of the epidemics have been attributed to *N. meningitidis* serogroup A (NmA) but some countries have also experienced NmX and NmW epidemics [4,5]. Burkina Faso, a west-African country of approximately 16 million inhabitants is one of the most affected countries and NmA of sequence types (ST)-5, ST-7 and ST-2859 have been successively responsible for most of the outbreaks [4,6]. In 2002, Burkina Faso was the first African country to experience a serogroup W epidemic, due to the hypervirulent ST-11 clone probably imported from Saudi Arabia [7-10]. In 2006 a major serogroup X epidemic caused by ST-181 affected the neighbouring country Niger [11,12] and the clone later spread to Burkina Faso [13].

Polysaccharide (Ps) vaccines, currently used to stop on-going epidemics [14,15], have not been successful in preventing new outbreaks as they provide limited and relatively brief protection in young children, do not induce immunological memory and do not protect against carriage [16]. Conjugate vaccines are immunogenic in young children and generate immunological memory. The conjugate vaccines' ability to prevent carriage acquisition is also another major advantage, as it reduces the transmission of the bacterium.

A new NmA polysaccharide-tetanus toxoid conjugated vaccine, MenAfriVac, developed to eliminate NmA epidemics in the meningitis belt, was first introduced on a national scale in Burkina Faso [17-21]. A pilot introduction in the district of Kaya was achieved in September 2010, followed by the vaccination of the remaining 1-29-year-olds in Burkina Faso in

December 2010 [21]. The rate of meningococcal disease and carriage was substantially reduced and herd immunity was generated [22,23]. One of the concerns when introducing a monovalent vaccine is the possibility for serogroup replacement or capsule switch [24-27]. Capsule switch from C to B after vaccination with a C conjugate vaccine has been documented in several countries [28-32] and the importance of molecular methods in post-vaccination surveillance has been emphasized [29,32,33].

To better understand the impact of implementing a monovalent meningococcal conjugate vaccine in the meningitis belt, including the potential for capsule switching, and to follow the evolution of circulating meningococcal isolates, we characterized invasive and carriage isolates collected in Burkina Faso in 2010 and 2011, before and after MenAfriVac introduction. We present here the molecular characteristics of circulating meningococci up to one year after vaccination.

# Methods

## Ethics

The collection of oropharyngeal samples in Burkina Faso was approved by the Norwegian Regional Committee for Medical Research Ethics, Southern Norway, the Ethical Committee for Health Research in Burkina Faso and the Internal Review Board at Centers for Disease Control and Prevention (CDC), Atlanta, USA. The collection of invasive isolates was performed within the Burkina Faso national healthcare system as part of the national surveillance and did not require ethical clearance.

## Meningococcal isolates from healthy carriers

In a repeated cross-sectional study performed in 2010 and 2011 oropharyngeal swabs were collected from a representative proportion of 1-29-year-old residents of three health districts in Burkina Faso; the urban district of Bogodogo, and the rural districts of Dandé and Kaya, during five sampling campaigns, named S5-S9, as described [22,34]. During each of the campaigns, > 5000 individuals aged 1- 29-years were enrolled within a 4-week period, yielding a total of 25,521 samples. Due to an earlier vaccination with MenAfriVac in the district of Kaya (September 2010) than in the rest of the country (December 2010), 3428 of the samples were collected from unvaccinated districts.

Swabbing and laboratory analysis aiming at identifying meningococci were performed by the staff of microbiological laboratories in Burkina Faso: the Centre Hospitalier Universitaire Pédiatrique Charles de Gaulle, Ouagadougou for Bogodogo; the Centre Hospitalier Universitaire Souro Sanou, Bobo-Dioulasso for Dandé; and the Centre Hospitalier Universitaire Yalgado, Ouagadougou for Kaya, as previously described [34]. Isolates suspected to be *N. meningitidis* were sent to Norwegian Institute of Public Health (NIPH) in

Oslo, Norway for confirmatory analysis and molecular characterization. All the isolates confirmed as *N. meningitidis* were subject to molecular characterization with the exception of 842 serogroup X isolates collected in the district of Kaya. Due to very high carriage rates of serogroup X meningococci in Kaya about one fourth (273/1115) of the serogroup X isolates were randomly selected for molecular characterization (range, 15.1 - 52.5% per campaign; Table 1).

### **Invasive meningococcal isolates**

Cerebrospinal fluid (CSF) samples were collected from 42 cases of meningitis in Burkina Faso, as part of the normal case investigation. CSF samples from the epidemic season of 2010 were sent to the WHO Collaborating Centre for Reference and Research on Meningococci at NIPH in Trans-isolate medium [35]. Samples from 2011 were first cultivated at the Centre Hospitalier Universitaire Pédiatrique Charles de Gaulle, Ouagadougou and meningococci were sent frozen on dry-ice.

### **Characterization of meningococcal isolates**

Standard laboratory methods were used to identify meningococci [34] and the serogroup was primarily determined by slide agglutination (Remel, GA, USA). DNA was isolated by suspending 1 loop of bacteria in 200 µl Tris-EDTA (TE) buffer, pH 8.0, heating at 95°C for 10 minutes and centrifugation at 16,000 x g for 5 min. Capsule gene PCR [36] was applied for serogroup determination of isolates non-serogroupable by slide agglutination. Multilocus sequence typing (MLST) using seven housekeeping gene fragments [37] was performed with oligonucleotide primers, as recommended on the MLST website (<http://pubmlst.org/neisseria/>). Classification by outer membrane protein PorA and FetA variants was done by DNA sequencing of the *porA* and *fetA* genes [38,39]. New MLST

alleles, sequence types (STs), PorA and FetA variants were submitted to the MLST database (<http://pubmlst.org/neisseria/>). For culture-negative CSF-samples, the genotypic characterization was limited to capsule gene PCR and *porA* sequencing following a nested *porA* -PCR [40].

The invasive isolates were tested for antibiotic susceptibility by determination of minimal inhibitory concentrations (MIC) of penicillin G, ciprofloxacin, ceftriaxone, rifampicin, tetracycline, chloramphenicol and sulphonamides using Etest (AB Biodisk, Solna, Sweden). The isolates were classified as susceptible, intermediate or resistant according to the breakpoints from the European Committee on Antibiotic Susceptibility Testing ([www.eucast.org](http://www.eucast.org)).



## Results

### Sample collection

A total of 1,659 meningococcal carriage isolates were retrieved, of which 112 came from unvaccinated districts in 2010 and 1,547 from vaccinated districts in 2010-2011. From a total of 1,205 isolates originating from Kaya, 1,115 were NmX and of these, a subset of 273 isolates was genotyped (Table 1). All the isolates from Bogodogo (158) and Dandé (296) and all the non-NmX isolates from Kaya (90) were subject to genotypic characterization. Hence, the total number of carriage isolates genetically characterized was 817. The number of characterized isolates varied by campaign and site (Table 2).

A total of 42 invasive isolates from Burkina Faso were sent to NIPH, 24 from 2010 before the vaccination campaign and 18 from 2011. Of these, 28 were culture-positive and could be analyzed by MLST, *porA* and *fetA* sequencing. The remaining 14 samples were tested by capsule gene PCR and *porA* sequencing.

### Molecular characterization of carriage isolates

The 817-strain collection subjected to molecular characterization was composed of 407 NmX (49.8 %), 220 NmY (26.9%), 102 NmW (12.5%), 78 (9.5%) nonserogroupable Nm (NmNG), 5 NmC (0.6%), 4 NmA (0.5%) and a single isolate of NmB (Table 3). The isolates were assigned to 28 different STs of which 20 belonged to 11 defined ST-complexes (Additional file 1). We identified 4 new alleles for the housekeeping genes included in the MLST scheme, 8 new STs, 5 new PorA variants and 4 new FetA variants, all submitted to the MLST database (<http://pubmlst.org/neisseria/>).

The strain collection was dominated by ST-181 of the ST-181 clonal complex, with 396 (48.5%) isolates (Additional file 1). The ST-181 isolates were serogroups X (n= 388) or NmNG (n=8). The dominant PorA/FetA combination of the ST-181 isolates was P1.5-1,10-1; F1-31 (91.0%), independently of the serogroup. In Kaya, 98.1% of the 273 genotyped NmX isolates were assigned to ST-181, 1.5% to ST-5789 and 0.4% (one isolate) to ST-9359. Both ST-5789 and ST-9359 are single locus variants (SLV) of ST-181 at the *adk* and *abcZ* loci, respectively. When extrapolating these results to all the 1115 NmX isolates from Kaya we considered that as many as 1094 NmX isolates from that district were probably ST-181. With 58 isolates from Bogodogo and 62 from Dandé the total number of carriers of NmX ST-181 was probably 1214, representing 73.2 % of the carriage isolates.

ST-4375 of the ST-23 complex was the second dominating ST representing 15.9% of the genotyped isolates (Additional file 1), 129 NmY and 1 NmNG. The ST-23 clonal complex was also represented by a single strain assigned to ST-9353, a SLV of ST-4375 in the *adk* locus first identified in this study. Within the ST-23 complex, the dominating PorA/FetA combination was P1.5-1,2-2;F5-8 (96.9%) independently of ST and serogroup.

Isolates assigned to ST-2881 of the ST-175 complex represented 9.2% of the isolates and were serogrouped as W (n=52), Y (n=12) or NG (n=11) (Additional file 1). Among the ST-2881 isolates, 84% expressed the PorA variant P1.5-1,2-36, 92% the FetA variant F5-1 and 76% the combination P1.5-1,2-36; F5-1. The ST-175 complex was also represented by the ST-8638 expressing either a W (n=4) or Y (n=1) capsule. All the ST-8638 isolates were characterized as P1.5-1,2-36; F5-1.

ST-767 of the ST-167 complex was represented by serogroups B (n=1), X (n=1), Y (n=56), and NmNG (n=4) meningococci and accounted for 7.6% of the genotyped strain collection

(Additional file 1). The PorA/FetA combination P1.5-1,2-2;F5-8 was expressed by 88.7% of the ST-767 isolates and 15 out of 16 isolates assigned to the two other STs belonging to the same clonal complex, the ST-2880 and the ST-7375.

A total of 42 (5.1%) ST-11 isolates were found, of which 41 were NmW and one was NmNG (Additional file 1). The ST-11 complex was also represented by two ST-9358 isolates identified in the district of Dandé during the sampling campaign S8. ST-9358 is a SLV of ST-11 in the *abcZ* locus. PorA variant P1.5,2 was expressed on the outer membrane of all the ST-11 complex isolates, Fet A variant F1-1 on 95.6 % of them and F5-4 on the remaining two isolates. ST-11 represented 40.6 % of the NmW isolates while 50.5% NmW were assigned to ST-2881. As opposed to the other major STs, ST-11 of the ST-11 complex was only present in the districts of Bogodogo and Dandé.

NmA carriage was only found in Dandé before vaccine introduction. A total of 4 isolates were identified and they all were of genotype ST-2859;P1.5-1,2-2;F3-1. None of the isolates assigned to ST-2859 or classified as P1.5-1,2-2 or F3-1 expressed any other serogroup than A.

### **Sequence type distribution of carriage isolates over time**

Only genotype ST-2859, expressing a serogroup A capsule, was present in a non-vaccinated district in October-November 2010, but was not found after the introduction of the serogroup A conjugate vaccine (Fig. 1). The other two genotypes known to cause outbreaks in Sub-Saharan Africa, NmX ST-181 and NmW ST-11 were both present after vaccine introduction. However, NmX ST-181 was circulating already since 2009 [34], while NmW ST-11 was detected only after vaccine introduction (Fig. 1). The ST-11 clone was first detected in the district of Dandé from S7 and then in Bogodogo from S8.

The two dominant genotypes of NmW isolates were ST-11 and ST-2881, but their distribution was not constant. ST-11 carriage peaked during S7 while ST-2881 peaked in S8 (Fig. 2).

### **Molecular characterization of invasive isolates**

The invasive isolates belonged to 5 STs (Table 4). All the NmA isolates were ST-2859;P1.20,9;F3-1. A single serogroup A isolate from a non-vaccinated 10-year old child was found after vaccine introduction. The NmX isolates were all ST-181;P1.5-1,10-1:F1-31 or F5-69. Fourteen culture-negative samples were P1.5-1,10-1 and likely represented the same NmX clone. Before introduction of MenAfriVac, a single NmW was analysed and characterized as ST-8638; P1.5-1,2-36; F5-1 of the ST-175 clonal complex. After vaccine introduction, all the NmW isolates were ST-11; P1.5,2;F1-1.

When comparing the genotypes of carriage isolates with that of invasive isolates, we found that the clones responsible for disease were the dominant genotypes of the corresponding serogroups among the carriage isolates; ST-2859;P1.5-1,2-2;F3-1 for NmA, ST-181;P1.5-1,10-1 ;F1-31 for NmX, and ST-11:P1.5,2;F1-1 for NmW.

### **Antibiotic susceptibility of invasive isolates**

All the 28 culture positive isolates were susceptible to ciprofloxacin (MIC range, 0.002 - 0.008), ceftriaxone (MIC range, <0.002 - 0.003), rifampicin (MIC range, 0.004 - 0.19), and chloramphenicol (MIC range, 0.5 - 2). Five serogroup W isolates were of intermediate resistance to penicillin G, all the remaining isolates were susceptible. The serogroup A isolates were resistant to tetracycline (MIC range, 3 - 4) while the other serogroups were not

(MIC range, 0.125 - 0.19). All serogroup A and W isolates were resistant to sulphonamides (MIC 24 - >1024).

## Discussion

We here present the molecular epidemiology of meningococcal carriage isolates collected in three districts in Burkina Faso immediately before and up to 13 months after the introduction of a serogroup A conjugate vaccine, in comparison with isolates recovered from patients in 2010 and 2011. Our study showed that the relatively high incidence of NmX disease in 2010 - 2011 was caused by the same clone (ST-181) found among carriers and patients before vaccination, while the hypervirulent NmW ST-11 clone seemed to have been reintroduced in Burkina Faso after mass vaccination. We did not find evidence that the NmA ST-2859 clone underwent a capsule switch up to 13 months after vaccination either in isolates from carriers or patients.

The strain collection used to compare genotypes of carriage and invasive isolates is a subset of the strains collected through national surveillance. Of 6732 suspected cases of meningitis reported in Burkina Faso in 2010, only 467 CSF samples were analyzed; 130 samples were Nm [41] and of those, 24 isolates sent to the WHO reference laboratory in Oslo. As a result of improved surveillance [23,42], of the 3875 cases reported in 2011, as many as 3125 CSF samples were analyzed; 257 samples were Nm and of those, 18 Nm isolates were sent to the WHO reference laboratory. Although the strain collection is not completely representative for the meningococcal disease epidemiology in Burkina Faso, it provides a valuable source of information, supplementing other research studies in the same area [13]. Furthermore, the high number of CSF samples analyzed at national laboratories in 2011 provides a better basis than in any previous year to study the disease-causing pathogens.

The genetic diversity of carriage isolates was low and comparable to that found in 2009 [34], as 96% of the isolates in both studies were assigned to only 11 different STs. Low genetic

diversity of meningococcal carriage isolates has been found also in other African countries [43-45], while the diversity is higher in Europe [46].

In the post-vaccination period up to 13 months after MenAfriVac vaccination, the most striking events were the disappearance of NmA ST-2859, the dominance of NmX ST-181 and the re-emergence of NmW ST-11. Our results show that the NmA isolates circulating in unvaccinated districts in October-November 2010 were identical to those circulating in 2009 [34] and those responsible for NmA disease since 2003 [4,5]. The significant reduction of NmA carriage and disease in 2011 has been attributed to the mass vaccination with a serogroup A conjugate vaccine [22,23] and this positive effect regarding disease continued in 2012 [41].

In 2009 NmX carriage prevalence in Burkina Faso was 0.44% and the majority of isolates were assigned to ST-181 [13]. NmX carriage was almost exclusively detected in the eastern districts of Bogodogo and Kaya with a significant increase of prevalence in Kaya during the epidemic season [13]. The strong increase of NmX ST-181 among carriers and patients in all three districts in 2010 and 2011 and a particularly high carriage prevalence of this clone in Kaya is consistent with a spread from the east to the west after an outbreak in Niger in 2006 [11]. The significant increase of post-vaccination carriage has been shown to be independent of MenAfriVac vaccination as both NmX carriage and disease increased in 2010 before vaccine introduction [22,23]. We here confirm that the increase was due to the ST-181 clone already circulating in 2009 [34].

Our study showed that, after vaccine introduction NmW ST-11 re-emerged among carriers as well as patients. The carriage prevalence of NmW in Burkina Faso in 2009 was 0.34% and all

the isolates belonged to the ST-175 cc; the majority assigned to ST-2881 [34]. During the sampling campaign S5 in October-November 2010 NmW was carried only in Bogodogo and Dandé and all the isolates were still ST-2881. Molecular characterization of invasive isolates in 2009 and 2010, before vaccination, was consistent with the carriage study: the only NmW isolate analyzed among the 8 NmW identified in Burkina Faso in 2010 [41] was assigned to ST-175cc;ST-8638 (Table 2). The first reappearance of NmW ST-11 in Burkina Faso was after the country-wide mass vaccination with MenAfriVac and it was identified in both carriage isolates and invasive isolates. Before this, the ST-11 cc was last seen in Burkina Faso in 2006 [5]. Although NmW ST-2881 has been reported to cause meningococcal disease [13,45], until now, the potential to cause large meningococcal outbreaks has been associated with the hypervirulent ST-11 clone; as it did in Burkina Faso in 2002 [9]. The re-emergence of ST-11 during the 2011 epidemic season and a shift towards carriage of ST-2881 after the epidemic season (Fig. 2) explains the increase and stabilization of post- vaccination NmW carriage reported earlier [41,47].

The circulation of NmW ST-11 exclusively in the districts of Bogodogo and Dandé might be related to the very high carriage prevalence of NmX in Kaya, where up to 23.6% of the individuals were NmX carriers [22]. In view of this clonal situation, it seems that NmW ST-11 was not able to compete for the same ecological niche in this district, while it succeeded in the other two districts with lower NmX carriage prevalence [22]. As ST-11 was first recovered in Dandé in February-March 2011 and then in Bogodogo in May, the re-introduction of ST-11 probably happened in the western part of the country, possibly from Mali as the Dandé district is close to Mali. The same NmW ST-11 clone caused disease in Mali in 2007 and 2009 [5,48]. Surveillance data from 2012 shows that the incidence of NmW disease further increased in Burkina Faso and other countries in the meningitis belt, including



those having introduced MenAfriVac [41]. Meningococcal disease caused by the same NmW ST-11 clone was also identified in France among people traveling from Senegal [47].

It can be speculated that the change in epidemiology from A to W disease might be attributed to a serogroup replacement caused by the major reduction of NmA carriage and disease incidence. However, pre-vaccination NmA carriage was extremely low [34], thus vaccine-induced serogroup replacement is unlikely to explain the findings. Epidemic waves have earlier shown to radically change the epidemiology in many countries [49]. The last NmW outbreak in Burkina Faso was 10 years ago and the re-emergence of this serogroup fits well with the cyclic reappearance of larger epidemics [8,41]. However, this evolution highlights the need for effective multivalent vaccines in the meningitis belt. Presently, it seems that a trivalent A+ W+X conjugate vaccine would have a broad coverage. Since invasive meningococcal clones in Africa are highly conserved, vaccines based on sub-capsular antigens would be an alternative or supplement to conjugate vaccines.

One concern when introducing a monovalent vaccine is that virulent isolates expressing the capsule targeted by the vaccine may take up genes coding for another capsule by horizontal gene transfer and evade the immune system [30,31,50]. Capsule switched isolates have been shown to conserve their virulence [29]. However, up to 13 months after MenAfriVac vaccination, we did not observe any capsule switch among the ST-2859 isolates studied. It seems that clones belonging to the ST-5 cc have been particularly adapted to the African population with ST-5, ST-7 and ST-2859 as the dominant disease causing genotypes since the late 1980's, nearly all expressing serogroup A, P1.20,9 and F3-1 [4-6]. From an evolutionary standpoint, the ST-2859 meningococcus would benefit from changing its capsule to adapt to the selective pressure added by this new vaccine. From the human perspective, a ST-2859

clone switching to a serogroup B or X capsule would be disastrous as adequate vaccines are neither available nor affordable in Sub-Saharan Africa. However, because the conjugate vaccine affects transmission of the clone [22] the likelihood that the virulent clone would acquire capsule genes from another meningococcal isolate is decreased. As capsule switch might occur any time after vaccine introduction, it is important to continue with the molecular characterization of isolates retrieved from national surveillance and carriage studies.

## Conclusion

In this study we did not find evidence of capsule switching after mass vaccination with MenAfriVac in Burkina Faso in 2010, but found that the increase of NmX carriage and disease in 2010 and 2011 was due to ST-181 and that the NmW ST-11 clone was re-introduced in 2010. Based on these events and the low pre-vaccination carriage prevalence of NmA [34], we believe the successive clonal waves of ST-181 and ST-11 have contributed to the increase of NmX and NmW disease [41] rather than a significant decrease in NmA disease and carriage [22,23]. After a successful implementation of MenAfriVac throughout the whole meningitis belt, the time of devastating NmA epidemics will hopefully be over. However, continued surveillance of disease and carriage and molecular characterization of meningococcal isolates is needed to monitor the long-term impact of vaccination, follow the genetic evolution and antibiotic susceptibility of meningococci, and to improve vaccine strategies. Further development of effective and affordable vaccines against the emerging serogroups X and W should be prioritized. The polysaccharide-conjugated vaccine approach has been successful but other vaccine strategies should also be explored. We here confirm the low genetic diversity of meningococcal disease-causing isolates in sub-Saharan Africa and the stability of sub-capsular antigen composition over time, suggesting that such antigens could be considered in future vaccine formulations, in addition to conjugate vaccines.

## **Competing interest**

The authors declare that they have no competing interests.

## **Authors' contribution**

PAK, IS, RO, LS, FD, DK, JDT, TAC, MLF and DAC participated in the design of the study. AKB, IS, ASO, SN, RO, LS were responsible for collecting carriage isolates. RO and DK provided the clinical isolates. PAK and FD were responsible for coordination of the carriage study. PAK and JDT contributed with training and supervision. PAK analyzed the data and drafted the manuscript. DAC conceived the study and was responsible for the molecular analysis. All the authors revised the manuscript and approved the final version

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### **Additional files**

**Additional file 1.xls: Molecular characteristics of 817 *N. meningitidis* strains colonizing 1-29-year-olds in Burkina Faso in 2010-2011.**

**Table 1: Selection of NmX carriage isolates from the district of Kaya for molecular characterization**

<b>Sampling campaign</b>	<b>S5</b>	<b>S6</b>	<b>S7</b>	<b>S8</b>	<b>S9</b>	<b>Total</b>
Total no. of NmX isolates in Kaya	396	286	219	120	94	1115
No. of NmX isolates selected for genotyping	80	50	33	62	48	273
Proportion (%) of NmX isolates selected for genotyping	20.2	17.5	15.1	52.5	51.6	24.2

**Table 2: Number of isolates subjected to molecular characterization by district and sampling campaign**

<b>Sampling campaign</b>	<b>S5</b>	<b>S6</b>	<b>S7</b>	<b>S8</b>	<b>S9</b>	<b>Total</b>
District						
Bogodogo	49 <sup>a)</sup>	22	38	31	18	158
Dandé	63 <sup>a)</sup>	72	48	71	42	296
Kaya	103	72	48	80	60	363
Total	215	166	134	182	120	817

<sup>a)</sup> Unvaccinated district at the time of S5

**Table 3: Number of genotyped carriage isolates from Burkina Faso before and after MenAfriVac vaccination, by serogroup.**

District	Pre-vaccination		Post-vaccination			Total
	B	D	B	D	K	
Serogroup						
A	0	4	0	0	0	4
B	0	0	0	0	1	1
C	2	0	1	0	2	5
W	5	5	23	58	11	102
X	29	21	42	42	273	407
Y	5	31	16	114	54	220
NG	8	2	27	19	22	78
Total	49	63	109	233	363	817

Isolates were obtained from the districts of Bogodogo (B), Dandé (D) and Kaya (K).

NG, nonserogroupable.



**Table 4: Molecular characteristics of 42 invasive *N. meningitidis* strains recovered from Burkina Faso in 2010-2011.**

Year	Culture	Serogroup	CC	ST	PorA	FetA	No. of isolates
2010	Pos.	A	5	2859	P1.20,9	F3-1	2
	Pos.	W	175	8638	P1.5-1,2-36	F5-1	1
	Pos.	X	181	181	P1.5-1,10-1	F1-31	6
	Pos.	X	181	181	P1.5-1,10-1	F5-69	1
	Neg.	ND	ND	ND	P1.5-1,10-1	ND	14
2011	Pos.	A	5	2859	P1.20,9	F3-1	1
	Pos.	W	11	11	P1.5,2	F1-1	7
	Pos.	X	181	181	P1.5-1,10-1	F1-31	4
	Pos.	X	181	181	P1.5-1,10-1	F5-69	6

CC, Clonal Complex ; ST, Sequence type ; Pos., Culture positive; Neg., Culture negative ; ND, not determined

## FIGURE LEGENDS

**Fig. 1: Genotypes of meningococcal carriage and invasive isolates from Burkina Faso before and after MenAfriVac vaccination.**

The figure represents the time points when different sequence types (ST) of carriage isolates (dark grey) and invasive isolates (red) were detected in the carriage study or national surveillance [41], respectively. Each ST was either detected or not detected (blank), or data was unavailable (grey). Horizontal lines separate ST complexes. The vertical line represents vaccination: during sampling campaign S5, the districts of Bodogogo (B) and Dandé (D) were not yet vaccinated while the Kaya district (K) had introduced the vaccine. UA, unassigned to any ST complex.

**Fig. 2: Number of *Neisseria meningitidis* serogroup W ST-11 and ST-2881 carriage isolates identified in Burkina Faso during the sampling campaigns S6-S9, 2010-2011.**

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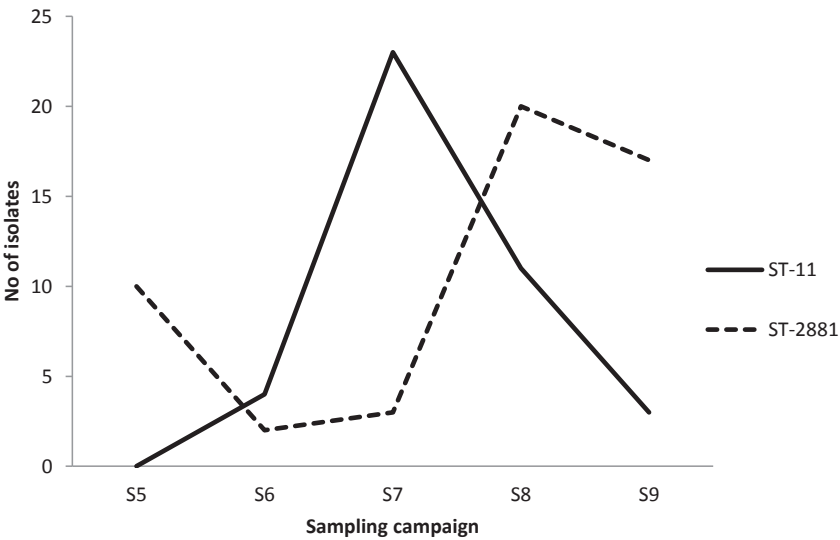
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[illegible]



Figure 2



Serogroup	ST-complex	ST no	PorA	FetA	No. of isolates
A	5	2859	P1.20,9	F3-1	4
B	167	767	P1.5-1,10-8	F1-31	1
C	41/44	206	P1.7-43,30	F5-121	2
C	41/44	206	P1.7-2,30-11	F5-2	1
C	865	865	P1.5-1,2-2	F1-6	2
W	11	11	P1.5,2	F1-1	40
W	11	11	P1.5,2	F4-5	1
W	11	9358	P1.5,2	F1-1	2
W	175	2881	P1.5-1,2-36	F5-1	41
W	175	2881	P1.5-1,2-36	F4-28	6
W	175	2881	P1.5-1,2-74	F5-1	4
W	175	2881	P1.5-1,10-1	F5-1	1
W	175	8638	P1.5-1,2-36	F5-1	4
W	175	9357	P1.5-1,2-36	F5-1	2
W	178	188	P1.19,15	F3-9	1
X	162	162	P1.7-2,4	F5-9	1
X	167	767	P1.5-1,10-8	F1-3	1
X	181	181	P1.5-1,10-1	F1-3	1
X	181	181	P1.5-1,10-1	F1-31	346
X	181	181	P1.5-1,10-1	F1-62	2
X	181	181	P1.5-1,10-1	F4-23	3
X	181	181	P1.5-1,10-1	F4-28	1
X	181	181	P1.5-1,10-1	F4-5	1
X	181	181	P1.5-1,10-1	F4-6	1
X	181	181	P1.5-1,10-1	F5-69	26
X	181	181	P1.5-1,10-1	F5-8	1
X	181	181	P1.5-1,10-1	F5-88	1
X	181	181	P1.5-1,10-1	F5-122	1
X	181	181	P1.5-1,10-1	F5-123	1
X	181	181	P1.5,2	F1-31	1
X	181	181	P1.5-1,2-2	F1-31	1
X	181	181	P1.5-3,10-1	F1-31	1
X	181	5789	P1.5-1,10-1	F3-3	1
X	181	5789	P1.5-1,10-1	F4-23	15
X	181	9359	P1.5-1,10-1	F1-31	1
Y	167	767	P1.5-1,10-8	F1-3	51
Y	167	767	P1.5-1,10-8	F1-31	1
Y	167	2880	P1.5-1,10-8	F1-3	4
Y	167	7375	P1.5-1,10-1	F1-3	1
Y	167	7375	P1.5-1,10-8	F1-3	11
Y	175	2881	P1.5-1,2-36	F5-1	10
Y	175	2881	P1.5-1,2-73	F5-1	2
Y	175	8638	P1.5-1,2-36	F5-1	1
Y	23	4375	P1.5,2	F5-8	1
Y	23	4375	P1.5-1,2-2	F5-8	123
Y	23	4375	P1.5-1,2-2	F5-28	1

Y	23	4375	P1.5-1,ND	F5-8	1
Y	23	4375	Neg.	F5-8	3
Y	23	9353	P1.5-1,2-2	F5-8	1
Y	UA	192	P1.18-11,42-1	Neg.	1
Y	UA	7697	P1.18-11,42-1	Neg.	3
Y	UA	9367	P1.21-15,16	F1-7	1
NG	11	11	P1.5-2	F1-1	1
NG	167	767	P1.5-1,10-8	F1-3	2
NG	167	767	P1.5-1,10-8	F1-31	1
NG	167	767	P1.5-1,10-8	Neg.	1
NG	175	2881	P1.5-1,2-36	F5-1	6
NG	175	2881	P1.5-1,2-74	F5-1	5
NG	178	188	P1.19,15	F3-9	1
NG	181	181	P1.5-1,10-1	F1-31	7
NG	181	181	P1.5-1,10-1	Frameshift	1
NG	198	198	P1.18,25	F5-5	5
NG	198	198	P1.18,25-47	F5-5	5
NG	198	198	P1.ND,25	F5-5	1
NG	23	4375	P1.5-1,2-2	F5-8	1
NG	41/44	9354	P1.7-2,9	F1-5	1
NG	UA	192	P1.18-11,42	Neg.	2
NG	UA	193	P1.18-11,42-1	Neg.	11
NG	UA	194	P1.18-11,42-8	Neg.	3
NG	UA	195	P1.18-11,42-9	Neg.	1
NG	UA	1289	P1.22,14-25	F5-5	1
NG	UA	4899	P1.21-14,28-3	F5-66	8
NG	UA	6920	P1.22-11,15-34	F6-5	1
NG	UA	7697	P1.18-11,42-1	Neg.	9
NG	UA	7697	P1.18-11,42-10	Neg.	1
NG	UA	9355	P1.21-14,28-3	F1-3	1
NG	UA	9356	P1.21-14,28-3	F5-66	1

NG: Nonserogroupable

UA: Unassigned

ND: Not Determined

Neg: Negative

Frameshift: Frameshift mutation



V









# Molecular Characterization of Invasive Meningococcal Isolates from Countries in the African Meningitis Belt before Introduction of a Serogroup A Conjugate Vaccine

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## Abstract

**Background:** The serogroup A conjugate meningococcal vaccine, MenAfriVac, was introduced in mass vaccination campaigns in December 2010 in Burkina Faso, Mali and Niger. In the coming years, vaccination will be extended to other African countries at risk of epidemics. To document the molecular characteristics of disease-causing meningococcal strains circulating in the meningitis belt of Africa before vaccine introduction, the World Health Organization Collaborating Centers on Meningococci in Europe and United States established a common strain collection of 773 isolates from cases of invasive meningococcal disease collected between 2004 and 2010 from 13 sub-Saharan countries.

**Methodology:** All isolates were characterized by multilocus sequence typing, and 487 (62%) were also analyzed for genetic variation in the surface antigens PorA and FetA. Antibiotic susceptibility was tested for part of the collection.

**Principal Findings:** Only 19 sequence types (STs) belonging to 6 clonal complexes were revealed. ST-5 clonal complex dominated with 578 (74.8%) isolates. All ST-5 complex isolates were remarkably homogeneous in their PorA (P1.20.9) and FetA (F3-1) and characterized the serogroup A strains which have been responsible for most epidemics during this time period. Sixty-eight (8.8%) of the 773 isolates belonged to the ST-11 clonal complex which was mainly represented by serogroup W135, while an additional 38 (4.9%) W135 isolates belonged to the ST-175 complex. Forty-eight (6.2%) serogroup X isolates from West Africa belonged to the ST-181 complex, while serogroup X cases in Kenya and Uganda were caused by an unrelated clone, ST-5403. Serogroup X, ST-181, emerged in Burkina Faso before vaccine introduction.

**Conclusions:** In the seven years preceding introduction of a new serogroup A conjugate vaccine, serogroup A of the ST-5 clonal complex was identified as the predominant disease-causing strain.

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## Introduction

The meningitis belt of Africa was first described by Lapeyssonnie in 1963 [1] and was further defined by Molesworth and co-workers in 2002 [2]. It now encompasses parts or the whole of 25 countries south of the Sahara, stretching from Ethiopia in the east to Senegal in the west. Every year during the dry season the region is affected by outbreaks of meningococcal meningitis and large epidemics emerge unpredictably 8 to 10 years apart. Traditionally, serogroup A meningococci have been responsible for most of the endemic disease as well as for the large epidemics, but more recently serogroup W135 and serogroup X strains have also been involved [3–13]. Emergence and dissemination of a new strain

may lead to epidemics under certain environmental conditions. New strains that appeared and were disseminated during the annual Hajj pilgrimage have been linked to several epidemics [14].

The most severe epidemic of meningococcal meningitis experienced by Africa was in 1996, with more than 150,000 reported cases and 16,000 deaths [15–17]. It was caused by a serogroup A clone of *Neisseria meningitidis* assigned to sequence type (ST) -5 using multilocus sequence typing [18]. In 2002, Burkina Faso was the first country to experience a major serogroup W135 epidemic, with 13,000 reported cases, and 1,400 deaths [10]. A hypervirulent ST-11 clone was responsible for this epidemic [7].

Preventive vaccination with meningococcal polysaccharide vaccines was usually not attempted because of their relatively short protection and low immunogenicity in young children [19]. Thus, the World Health Organization (WHO) recommended reactive mass vaccination to halt epidemics using either the A/C or the A/C/W135 polysaccharide vaccines, depending on the serogroup of the outbreak strains [20].

Starting in 2002, the Meningitis Vaccine Project (MVP), a public-private partnership between WHO and the Program for Appropriate Technology in Health (PATH), developed an effective monovalent serogroup A conjugate meningococcal vaccine, MenAfriVac, at a price affordable for African countries, with the aim of eliminating the devastating serogroup A epidemics occurring in sub-Saharan Africa [21]. The vaccine was introduced in a country-wide mass vaccination campaign in Burkina Faso in December 2010, where the vaccine was offered to all individuals aged 1 to 29 years. Vaccination of the same age group started in Mali and Niger in 2010 and was completed in 2011 [22].

In 2005 the two WHO Collaborating Centers for Reference and Research on Meningococci in Marseille, France, and Oslo, Norway, published an overview on the phenotypic and genotypic features of meningococcal isolates recovered from meningitis cases between 1988 and 2003 in 13 countries of the African meningitis belt [18]. We report here the molecular characteristics of the strains circulating in these countries from 2004 to 2010, prior to MenAfriVac introduction. The strain collection established by these two WHO Collaborating Centers was supplemented with isolates received by the WHO Collaborating Center for Prevention and Control of Epidemic Meningitis, Centers for Disease Control and Prevention, Atlanta, GA, and by the Institut Pasteur, Paris, France, which is a newly nominated WHO Collaborating Center, following closure of the WHO Center in Marseille in 2010.

## Materials and Methods

### Bacterial isolates

The strain collection comprised a total of 773 isolates, all recovered from cerebrospinal fluid samples from patients of countries of the African meningitis belt and forwarded to the WHO Collaborating Centres. Strains from patients in other African countries were excluded, as were isolates from asymptomatic carriers. The sources of the isolates were as follows: 1) a total of 372 isolates from 2004 to 2009 constituted a large part (88%) of the collection of invasive isolates from the WHO Collaborating Centre in Marseille for that period; the isolates were transferred to the other WHO Collaborating Centres under contract for scientific purposes; 2) 360 isolates were from the WHO Collaborating Centre in Oslo. These were assembled either by physicians working for Médecins Sans Frontières, using Trans-Isolate media [23] to collect samples in areas where meningitis outbreaks were occurring; during field evaluations performed by the WHO Inter-country Support Team, Ouagadougou; or directly by the co-authors; 3) the remaining isolates were sent to the WHO Collaborating Centre by co-authors. Bacterial identification was determined by Gram staining, the oxidase reaction, and standard biochemical tests. The strains were stored at  $-80^{\circ}\text{C}$  in brain heart broth with 15% sterile glycerol or in Greaves solution [24].

### Serogrouping

*N. meningitidis* strains were serogrouped by slide agglutination with sera manufactured by the Institut de Médecine Tropicale du Service de Santé des Armées, Marseilles, France, or commercial antisera (Remel, GA, USA).

### Antimicrobial sensitivity testing

Antimicrobial susceptibility testing was performed by determination of the minimal inhibitory concentrations (MIC) using Etest (AB Biodisk, Solna, Sweden). Isolates were tested for susceptibility to penicillin G, amoxicillin, ceftriaxone, ciprofloxacin, chloramphenicol, rifampin, tetracycline and sulphonamides, and classified using the breakpoints from the European Committee on Antimicrobial Susceptibility Testing (<http://www.eucast.org/>).

### Genotypic characterization

DNA from each strain was prepared by suspending bacteria in Tris-EDTA buffer (10 mM Tris-HCl and 1 mM EDTA), pH 8.0, heating at  $95^{\circ}\text{C}$  for 10 min, and followed by centrifugation at  $16,000\times g$  for 5 min. The supernatant was used as DNA template for PCR. After DNA amplification by PCR, sequence of fragments from the seven housekeeping genes, *abcZ* (putative ABC transporter), *adk* (adenylate kinase), *aroE* (shikimate dehydrogenase), *fumC* (fumarate), *gdh* (glucose-6-phosphate dehydrogenase), *pdhC* (pyruvate dehydrogenase subunit), and *pgm* (phosphoglucotase), were analysed on an AB Prism 373, AB Prism 377 or AB 3130XL DNA sequencer (Applied Biosystems, Foster City, CA), according to the method on the MLST website (<http://pubmlst.org/neisseria/>). The DNA sequences were compared with the existing alleles on the MLST website using sequence query or the meningococcal genome informatics platform (MGIP) for determination of the allele numbers, STs, and clonal complexes of the isolates [25].

Variation in the *porA* and *fetA* genes, coding for the outer membrane proteins PorA and FetA, respectively, was determined by DNA sequencing, as described previously [26,27]. New MLST alleles and STs were submitted to the MLST database (<http://pubmlst.org/neisseria/>), together with the strain serogroup and *porA* and *fetA* sequences.

PCR analysis of the genes coding for the polysaccharide capsule was performed for genogroup determination of non-serogroupable isolates as described [28].

### Data analyses

All the data were entered into an Excel database (Microsoft Corporation, Redmond, WA) and analysed using R version 2.10.0 [29].

## Results

### Origin of isolates

The isolates analysed by MLST originated from Benin ( $n = 12$ ), Burkina Faso ( $n = 285$ ), Cameroon ( $n = 24$ ), Central African Republic (CAR) ( $n = 5$ ), Chad ( $n = 53$ ), Ghana ( $n = 6$ ), Kenya ( $n = 3$ ), Mali ( $n = 132$ ), Niger ( $n = 124$ ), Nigeria ( $n = 57$ ), Sudan ( $n = 22$ ), Togo ( $n = 29$ ) and Uganda ( $n = 21$ ). The number of isolates retrieved by country and year varied greatly, ranging from zero for some countries in some years to 128 from Burkina Faso in 2006 (Table 1). For Burkina Faso, Chad, Mali and Niger, meningococcal isolates were obtained and characterized for at least 6 of the 7 years of the period analysed.

### Serogroups

Of the 773 isolates, 568 (73.5%) were serogroup A, 105 (13.6%) serogroup W135, 63 (8.2%) serogroup X, 22 (2.8%) serogroup Y and 15 (1.9%) were non-groupable (NG). Serogroup W135 was present in all these 13 countries within the meningitis belt, while serogroup A was recovered from all countries except Kenya. Serogroup X was mainly found in Niger, Burkina Faso and Uganda. Burkina Faso was the only country where all the

**Table 1.** Serogroup and sequence type (ST) of invasive meningococcal isolates from sub-Saharan Africa in 2004–2010.

Year	Serogroup	Clonal complex	ST	No. of strains (yearly %)	Country source (no. of isolates)
2004	A	5	7	23 (32.4)	Benin (1); CAR <sup>c</sup> (4); Ghana (4); Niger (13); Nigeria (1)
	A	5	2859	17 (23.9)	Burkina Faso (16); Mali (1);
	W135	11	11	11 (15.5)	Benin (1); Burkina Faso (8); Ghana (2)
	W135	23	4375	1 (1.4)	Burkina Faso (1);
	W135	175	2881	3 (4.2)	Benin (1); Chad (1); Nigeria (1)
	X	181	181	3 (4.2)	Niger (3)
	Y	11	11	1 (1.4)	Burkina Faso (1)
	Y	23	4375	1 (1.4)	Burkina Faso (1)
	Y	167	767	1 (1.4)	Benin (1)
	NG <sup>a</sup>	5	2859	7 (9.9)	Burkina Faso (6); Mali (1)
	NG	23	4375	2 (2.8)	Burkina Faso (2)
	NG	UA <sup>b</sup>	192	1 (1.4)	Burkina Faso (1)
2005	W135	11	11	6 (35.3)	Chad (4); Kenya (2)
	W135	175	2881	4 (23.5)	Niger (4)
	X	181	181	7 (41.2)	Niger (7)
2006	A	5	7	36 (16.0)	Burkina Faso (1); Mali (1); Niger (25); Nigeria (3); Sudan (6)
	A	5	2859	126 (56.0)	Burkina Faso (122); Mali (4)
	A	5	5788	1 (0.4)	Niger (1)
	W135	11	11	14 (6.2)	Chad (1); Mali (5); Sudan (3); Uganda (5)
	W135	11	5779	1 (0.4)	Burkina Faso (1)
	W135	175	2881	5 (2.2)	Benin (3); Niger (2)
	X	181	181	23 (10.2)	Niger (23)
	X	181	5789	1 (0.4)	Niger (2)
	X	UA	5403	9 (4.0)	Kenya (1); Uganda (8)
	Y	167	2953	1 (0.4)	Benin (1)
	Y	23	4375	5 (2.2)	Burkina Faso (4); Niger (1)
	Y	167	767	2 (0.9)	Mali (2)
2007	A	5	7	34 (18.6)	Chad (4); Mali (3); Niger (12); Nigeria (3); Sudan (12)
	A	5	2859	113 (61.7)	Burkina Faso (71); Mali (23); Togo (19)
	W135	11	11	8 (4.4)	Mali (8)
	W135	175	2881	8 (4.4)	Benin (1); Chad (1); Togo (6)
	X	181	181	3 (1.6)	Burkina Faso (3)
	X	UA	5403	6 (3.3)	Uganda (6)
	Y	23	4375	1 (0.5)	Burkina Faso (1)
	Y	167	767	4 (2.2)	Mali (4)
	Y	167	2880	1 (0.5)	Burkina Faso (1)
	Y	167	8620	1 (0.5)	Mali (1)
	Y	UA	192	1 (0.5)	Mali (1)
	NG	5	2859	2 (1.1)	Burkina Faso (1); Mali (1)
	NG	UA	192	1 (0.5)	Burkina Faso (1)
2008	A	5	7	18 (16.8)	Mali (3); Niger (7); Nigeria (7); Sudan (1)
	A	5	2859	62 (57.9)	Burkina Faso (24); Mali (36); Niger (2)
	A	5	6968	1 (0.9)	Burkina Faso (1)
	W135	11	11	5 (4.7)	Cameroon (1); CAR (1); Chad (3)
	W135	175	2881	13 (12.1)	Benin (1); Burkina Faso (1); Cameroon (9); Togo (2)
	X	181	181	3 (2.8)	Benin (1); Togo (2)
	Y	23	4375	1 (0.9)	Burkina Faso (1)
	Y	167	767	2 (1.9)	Benin (1); Mali (1)
	NG	5	2859	1 (0.9)	Mali (1)

**Table 1.** Cont.

Year	Serogroup	Clonal complex	ST	No. of strains (yearly %)	Country source (no. of isolates)
2009	NG	11	11	1 (0.9)	Chad (1)
	A	5	7	60 (65.2)	Cameroon (1); Chad (1); Niger (21); Nigeria (37)
	A	5	2859	16 (17.4)	Burkina Faso (6); Mali (9); Niger (1)
	W135	11	11	12 (13.0)	Cameroon (6); Chad (3); Mali (1); Nigeria (2)
	W135	175	2881	4 (4.3)	Cameroon (4)
2010	A	5	7	36 (46.2)	Cameroon (1); Chad (29); Mali (3); Nigeria (1); Uganda (2)
	A	5	2859	24 (30.8)	Burkina Faso (2); Mali (22)
	A	5	8639	1 (1.3)	Burkina Faso (1)
	W135	11	11	8 810.3)	Cameroon (2); Chad (5); Nigeria (1)
	W135	11	8637	1 (1.3)	Nigeria (1)
	W135	175	8638	1 (1.3)	Burkina Faso (1)
	X	181	181	7 (9.0)	Burkina Faso (7)

<sup>a</sup>NG, Non-groupable as determined by slide agglutination method.<sup>b</sup>UA, Unassigned to any clonal complex.<sup>c</sup>CAR, Central African Republic.

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serogroups were isolated. No serogroup B or C isolate was recovered from any of the countries.

Genogrouping was performed on the 11 of the 15 NG isolates that were still available. The genes for serogroup A and serogroup Y capsule were detected in 5 and 2 isolates, respectively, but no PCR product was obtained with any of the primer sets for the remaining 4 isolates.

### Antibiotic susceptibility

Antimicrobial susceptibility testing was performed for a selection of isolates (Table 2). All isolates tested were susceptible to amoxicillin, ceftriaxone, ciprofloxacin, chloramphenicol and rifampin. Of the 336 isolates tested against sulphonamide, all but 9 were resistant; the exceptions were 8 serogroup X isolates from Burkina Faso and a serogroup W135 isolate from Kenya. Among the isolates tested against tetracycline, all serogroup A strains were resistant while all the serogroup W135 and X isolates were susceptible. Reduced susceptibility to penicillin was seen for 9% of the isolates, but there was no association with the serogroup of the strains.

### Molecular characterisation

The ST-5 clonal complex dominated the strain collection with 578 isolates (74.8%), followed by 68 (8.8%) isolates of ST-11 complex, 48 (6.2%) isolates of ST-181 complex, 38 (4.9%) isolates of ST-175 complex, 12 (1.6%) isolates of ST-23 complex and 11 (1.4%) isolates of ST-167 complex (Table 3). The remaining 18 isolates belong to a ST that cannot be assigned to any ST complex; ST-192 (3 isolates) and ST-5403 (15 isolates).

The dominating ST-5 complex was composed of 368 (63.7%) ST-2859 isolates, 207 (35.8%) ST-7 isolates, as well as one isolate (0.2%) of each of the STs 6968, 5788 and 8639. The isolates of the ST-5 complex were either serogroup A (568 isolates) or NG (10 isolates) and a total of 344 ST-5 complex isolates that were further subtyped had identical PorA (P1.20,9) and FetA (F3-1). All serogroup A isolates belonged to the ST-5 complex.

The ST-11 clonal complex included 66 (97%) ST-11 isolates and two SLVs of ST-11, ST-5779 and ST-8637, both with one isolate each. The ST-11 complex was mainly composed of serogroup W135 isolates (97%). All 53 W135 ST-11 complex isolates for which PorA was sequenced were P1.5,2. FetA variant

**Table 2.** Antibiotic susceptibility of invasive meningococcal isolates from sub-Saharan Africa in 2004–2010.

Antibiotic	No. of isolates tested	MIC range ( $\mu\text{g/ml}$ )	% of isolates		
			Susceptible	Intermediate	Resistant
Penicillin G	526	0.012–0.64	91	7	2
Amoxicillin	189	0.012–0.19	100	0	0
Ceftriaxone	364	0.001–0.004	100	0	0
Ciprofloxacin	336	0.003–0.008	100	0	0
Chloramphenicol	526	0.38–2	100	0	0
Rifampin	508	0.002–0.25	100	0	0
Tetracycline	169	0.125–8	20	0	80
Sulphonamide	336	0.025– $\geq 1024$	2	0	98

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**Table 3.** Distribution of lineages found among 773 *N. meningitidis* strains isolated in the African meningitis belt between 2004 and 2010.

Clonal complex	ST	Serogroup	No. (%) of isolates
ST-5	7	A	207 (26.8%)
	2859	A	358 (46.3%)
		NG <sup>a</sup>	10 (1.3%)
	5788	A	1 (0.13%)
	6968	A	1 (0.13%)
ST-11	8639	A	1 (0.13%)
	11	NG	1 (0.13%)
		W135	64 (8.28%)
		Y	1 (0.13%)
	5779	W135	1 (0.13%)
ST-23	8637	W135	1 (0.13%)
	2953	Y	1 (0.13%)
	4375	NG	2 (0.26%)
		W135	1 (0.13%)
		Y	8 (1.04%)
ST-167	767	Y	9 (1.17%)
	2880	Y	1 (0.13%)
	8620	Y	1 (0.13%)
ST-175	2881	W135	37 (4.79%)
	8638	W135	1 (0.13%)
ST-181	181	X	46 (5.95%)
	5789	X	2 (0.26%)
UA <sup>b</sup>	192	NG	2 (0.26%)
		Y	1 (0.13%)
	5403	X	15 (1.9%)

<sup>a</sup>NG, Non-groupable as determined by slide agglutination method.<sup>b</sup>UA, Unassigned to any clonal complex.

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F1-1 dominated within the ST-11 complex with 92.7% of sequenced isolates.

The other clonal complex associated with serogroup W135 was the ST-175 complex, with 37 isolates (97.4%) of ST-2881 and one isolate of ST-8638, a SLV of ST-2881 identified in Burkina Faso in 2010. The dominant PorA-FetA combination among the 20 sequenced ST-175 complex isolates was P1.5-1,2-36/F5-1 (85%).

The ST-181 clonal complex consisted of ST-181 (95.8%) and ST-5789 (4.2%), a SLV of ST-181 at the *adk* locus, first encountered in Niger in 2006. All these isolates were serogroup X. Of the 27 ST-181 complex isolates analyzed for PorA and FetA, all were P1.5-1,10-1, while FetA varied with 74% F1-31, 22% F4-23, and 4% F5-69. All 48 ST-181 complex isolates originated from West Africa: Niger (n = 35), Burkina Faso (n = 10), Benin (n = 1) and Togo (n = 2).

Serogroup X causing disease in East Africa (Kenya and Uganda) belonged to ST-5403, a clone unrelated to the ST-181 complex and not yet assigned to a clonal complex. Of the 15 ST-5403 isolates that were sequenced for *porA* and *fetA* genes, 14 were P1.19,26/F3-27; one was P1.19,26-4/F3-27.

The serogroup Y isolates were mostly represented by the ST-23 and ST-167 clonal complexes. The ST-23 complex comprised 11 isolates of ST-4375 originating from Burkina Faso (n = 10) and

Niger (n = 1), as well as one ST-2954 isolate from Benin. The ST-167 complex was represented by nine ST-767 isolates from Benin (n = 2) and Mali (n = 7), one ST-2880 isolate from Burkina Faso and one ST-8620 isolates from Mali. Both ST-2880 and ST-8620 are SLVs of ST-767, differing in the *aroE* and *fumC* loci, respectively. Ten of the ST-167 complex isolates were P1.5-1,10-8/F1-3 and one was P1.5-1,10-8/F3-1. All seven serogroup Y ST-4375 isolates analyzed were P1.5-1,2-2/F5-8.

### Temporal changes within countries

The disease-causing strains varied in individual countries of the meningitis belt within the reported time interval. For the three countries with most analyzed isolates, the serogroup distribution by year is shown in Fig. 1. In Burkina Faso, serogroup A dominated during the whole period except for 2010, when serogroups X, A and W135 were represented by 7, 2 and 1 strains, respectively. In Mali, serogroup A has been responsible for ~80% of cases in all the years, but in 2006 the proportion of serogroup A and serogroup W135 isolates was equal. In Niger serogroup A isolates dominated except for 2005–2006, when serogroup X prevailed. Unfortunately, we did not obtain any case isolates from Niger in 2010. However, an increase in isolates of serogroup W135 and a decline in serogroup X were reported in Niger in 2010 [4].

In Chad, serogroup W135 dominated during the whole period except for 2007 and 2010, when serogroup A was prevalent; but the numbers of isolates were small.

### Evolution of ST-5 complex

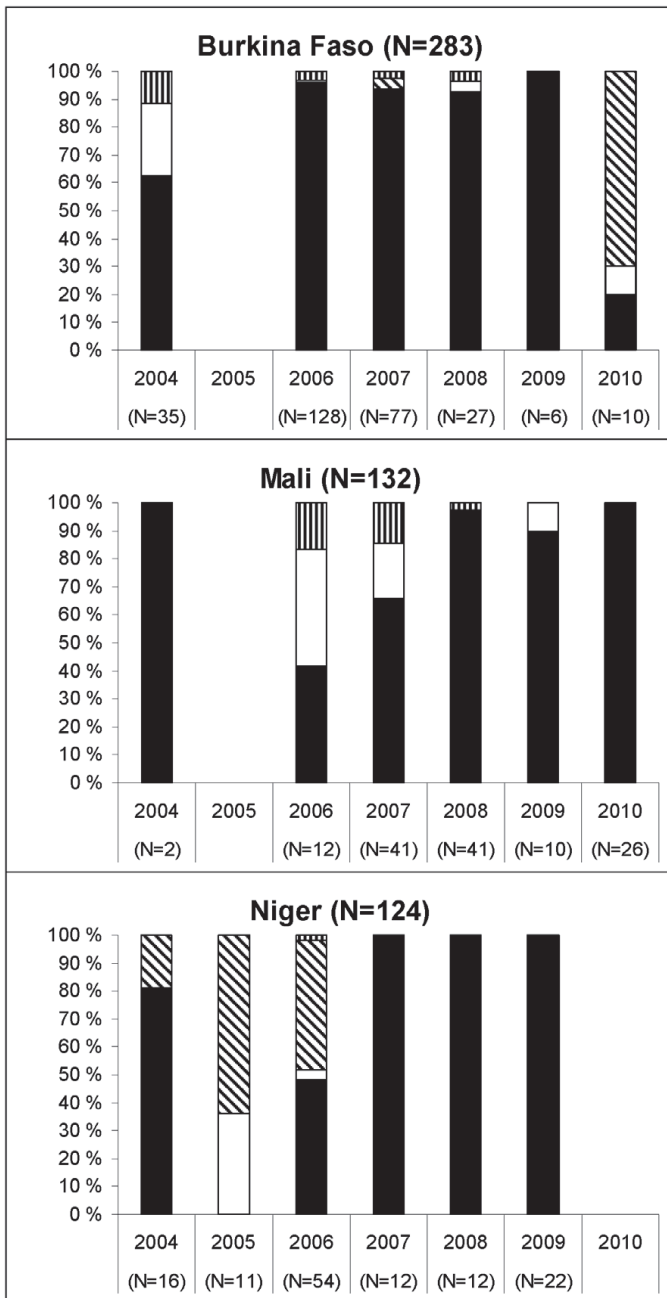
Since the introduction of the ST-5 complex in the meningitis belt in 1987 [9], variation in housekeeping genes has occurred, resulting in new STs (Fig. 2). In the period 2004–2010, ST-7, which had already totally replaced ST-5 in the meningitis belt by 2003 [18], and ST-2859, which emerged in Burkina Faso in 2003, dominated within the ST-5 complex. In addition, three new STs emerged during the period 2004–2010: ST-6968, a single locus variant (SLV) of ST-2859 in the *pdhC* locus was first identified in Burkina Faso in 2008; ST-5788 and ST-8639 were first identified in Niger in 2006 and Mali in 2010, respectively, and are SLVs of ST-7, in the *aroE* and *pdhC* loci, respectively. In contrast, no variation in the genes coding for the surface exposed outer membrane proteins PorA and FetA has been detected.

### Discussion

This study included 773 isolates recovered from meningococcal disease cases in 13 countries of the meningitis belt during a 7-year period. Only 19 distinct STs were revealed. These STs belonged to 6 clonal complexes, two of which, the ST-5 and the ST-11 complexes, accounted for 83.6% of the isolates.

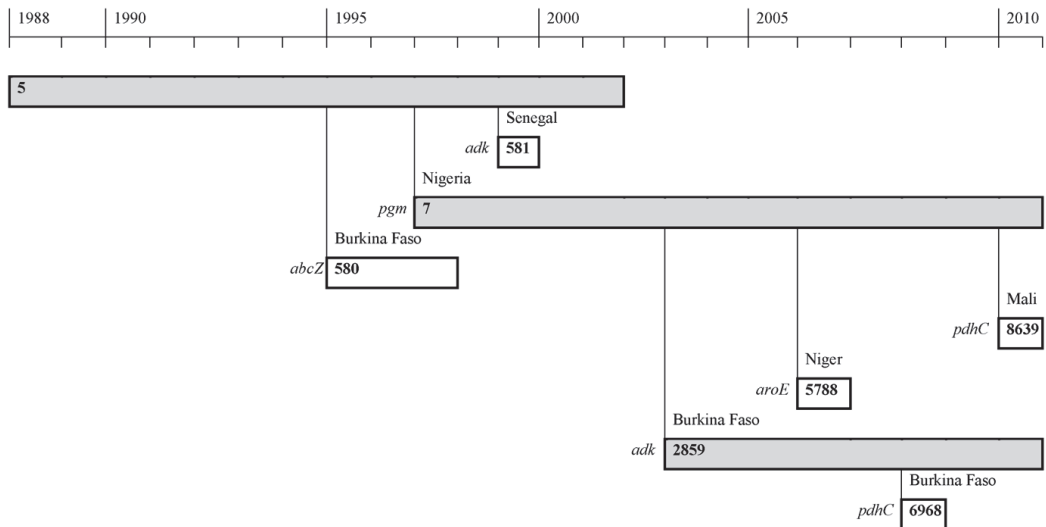
### Limitation of the study

In the period 2004 to 2010 approximately 225,000 meningitis cases from 12 countries within the African meningitis belt were reported by the WHO Inter country Support Team – West Africa, which compiles information from most countries of the sub-Saharan Africa [30]. This is summarized in Table 4 which showed a relatively quiet year in 2005 with less than 7,000 reported cases to the peak year of 2009 with nearly 80,000 reported cases. Of the reported meningitis cases less than 10% have a clinical specimen taken and examined microbiologically in the country of origin. Meningococcal meningitis was laboratory-confirmed for 7803 patients, i.e. about for one third of the patients from whom a clinical sample was analysed.



**Figure 1. Serogroup distribution (%) in Burkina Faso, Mali and Niger in the period 2004–2010.** In the figure, the few non-groupable isolates were included together with those assigned to a serogroup on the basis of the capsule PCR and/or their molecular profile, except for ST-192 isolates (n = 2) that were likely to harbour a capsule null gene [36]. Black bars: serogroup A; white bars: serogroup Y; oblique strips: serogroup X; vertical strips: serogroup Y.

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**Figure 2. Evolution of the ST-5 complex in the meningitis belt in the period 1988–2010.** The ST is shown within the bar, the country where the new ST was first detected is indicated above and the locus changed to the left. The left end of the bar shows when the variant was first detected and the right end shows the last reported isolation. The three dominant STs are marked with grey background.  
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We here report the molecular characterization of 773 isolates collected over this 7-year period. In our previous study, encompassing a period of 15 years, data on a total of 357 isolates had been collected [18]. This shows an overall improvement likely resulting from a better use of the laboratory in meningitis diagnosis in Africa and an increase networking of the WHO Collaborating Centers. However, in comparison to the huge number of cases occurring in the region, only a very small fraction of cases were represented in our analysis. Relating to the data collated by the WHO Inter country Support Team – West Africa the proportion of culture-confirmed meningococcal cases included in our study ranged from 4.2% in 2009 to 27.2% in 2007 (Table 4). The representation of the different countries was variable; especially we were unable to obtain strains from the Ivory Coast and Ethiopia.

From these two countries, however, only 32 and 51 cases, respectively, have been culture-confirmed in this 7-year period according to reported data [30]. From the remaining countries of the meningitis belt that report to the WHO Inter country Support Team – West Africa, our collection included from 2.5% of the laboratory-confirmed cases from Niger (124 of 5061 cases) and up to 70.6% of the laboratory-confirmed cases from Mali (132 of 187 cases). These data illustrate the great differences both in surveillance systems and laboratory-based diagnosis of meningococcal meningitis between countries of the belt.

Comparing our results for the 3 countries with most isolates (Fig. 1) with the serogroup distribution reported in the WHO Meningitis Weekly Bulletin, we see however similar proportions and trends [30]. In Burkina Faso, 1,090 meningitis cases were

**Table 4.** Number (%) of studied isolates in relation to the number of laboratory-confirmed cases and number of reported cases per year in countries of the meningitis belt.

Year	No. of reported cases	No. of CSF samples analysed	No. of laboratory-confirmed MD cases	No. of isolates in the study (%)
2004	31,712	NA <sup>a</sup>	809	71 (8.8)
2005	9,876	1,193	190	17 (8.9)
2006	41,526	6,215	1,572	225 (14.3)
2007	45,997	2,533	680	183 (26.9)
2008	33,381	3,413	1,134	107 (9.4)
2009	88,199	5,688	2,210	92 (4.2)
2010	30,103	4,132	1,238	78 (6.3)

Data source WHO Inter country Support Team – WestAfrica [30]. Numbers from the Democratic Republic of Congo have been removed to include only countries of the meningitis belt.

<sup>a</sup>NA, Information not available.

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laboratory-confirmed in-country for the 7-year period. The great majority of isolates were serogroup A, except for 2004 and 2010 when a mixed situation was seen. In 2004, the second serogroup was W135, while in 2010 serogroup X emerged toward the end of the season; these situations were evidenced both by the in-country surveillance and our strain collection.

Serogroup A dominated in Mali during the entire period. The mixed population we described in 2006 and 2007 was not evident from the WHO Meningitis Weekly Bulletin, where a few non-A cases were laboratory-confirmed (1 of 36 in 2006 and 5 of 60 in 2007) [30].

In Niger the National Reference Laboratory (CERMES) in Niamey provides a remarkable example of what kind of laboratory support can be achieved in West Africa. More than one thousand meningococcal meningitis cases have been confirmed and serogrouped during the most severe epidemic years of 2006 and 2009. The representativeness of the small fraction of isolates from Niger examined in this work is shown by the gradual increase in serogroup X until 2006, followed by a new wave of serogroup A cases in 2009.

For the other countries data are sporadic and our strain collection can only show which clonal complex and specific variant has been responsible for specific outbreaks.

### Dominance of serogroup A

Serogroup A *N. meningitidis* was responsible for most of the meningococcal outbreaks in 2004–2010, as it was for the period 1988–2003 [18]. The genetic diversity of serogroup A strains was low as all 568 isolates belonged to only five closely related STs of the ST-5 complex and all 344 isolates analyzed for PorA and FetA were P1.20,9/F3-1. Interestingly, this *porA-fetA* sequence combination has been stable among the serogroup A strains that cause disease in the meningitis belt for more than two decades, which suggests that the immunologic selection pressure on these outer membrane proteins is low. This is in contrast to the high variability identified in the genes coding for these two potential vaccine antigens among serogroup B isolates [31,32]. Serogroup A strains of the ST-5 complex from other geographical areas may also harbour the P1.20,9/F3-1 antigen combination, but variants, especially for FetA, are not uncommon (<http://pubmlst.org/neisseria/>). This observation should be considered when developing strategies for protein-based vaccines for Africa.

Indeed, SLVs and double locus variants were detected among serogroup A isolates belonging to the ST-5 complex, and some of them (ST-7 and ST-2859) were clearly successful in successive clonal replacement within the complex. This study confirms complete replacement of ST-5 with ST-7 that occurred after 2001 [18], as none of the strains from the period 2004–2010 were ST-5. However, during this period ST-2859 which was first discovered in Burkina Faso in 2003 completely replaced ST-7 in that country. No serogroup A isolates recovered from Burkina Faso since 2003 were ST-7. The total replacement of ST-7 by ST-2859 in Burkina Faso was also evidenced in a carriage study performed in 2009 when ST-2859 was identified as the only serogroup A clone circulating among healthy carriers [28]. ST-2859 has spread from Burkina Faso to Mali in 2004 and to Niger in 2008. In these two countries, however, replacement of ST-7 by ST-2859 has not been complete, as both clones were still recovered in Niger in 2009 and in Mali in 2010. ST-2859 has not been observed in the other countries of the meningitis belt. The severe epidemic in Nigeria in 2009 (more than 56,000 reported cases) was caused by ST-7.

### Continuous spread of serogroup W135

The first large epidemic caused by serogroup W135, ST-11, occurred in Burkina Faso in 2002, probably following amplification of this clone after the annual Hajj pilgrimages of 2000 and 2001 [7,33]. Prior to the Burkina Faso epidemic, serogroup W135 ST-11 isolates had been identified in Chad in 1996, Cameroon and Senegal in 2000, CAR in 2001, showing a large dispersion of the clone in the meningitis belt [18]. In the period 2004–2010, the ST-11 complex was identified in all the countries of the meningitis belt where isolates were obtained, except for Niger and Togo. While ST-11 complex was present in Niger in 2002 and 2003, from 2004 the W135 isolates recovered from that country belonged to ST-2881 of the ST-175 complex. In 2010, W135 predominated in Niger [30], but no isolates were available to us. Laboratory identification of the etiological agent was mainly performed by PCR, but all 9 W135 isolates tested by Collard and co-workers [4] belonged to ST-11. The ST-11 complex seemed to disappear also from Burkina Faso after 2006 and the few W135 recovered then belonged to the ST-175 complex. In Chad serogroup W135 dominated during most of the period 2004–2010, belonging either to ST-11 (2005, 2006, 2008–2010) or ST-2881 (2004 and 2007). In the years 2008–2010, the proportion of serogroup W135 in Chad decreased while the proportion of serogroup A increased (Fig. 1). In Niger, isolates of serogroup W135 belonging to ST-11 accounted for nearly 50% of the case isolates in 2010 [4]. In contrast to the ST-11 complex, the ST-175 complex has not been associated with major epidemics and is responsible for endemic disease cases. This clonal complex is geographically widespread and has been identified in meningococcal carriage studies in Europe and Asia (<http://pubmlst.org/neisseria/>).

### Emergence of serogroup X

Carriage and sporadic disease-associated serogroup X isolates have been detected in the meningitis belt as early as in the 1990s [6,8,34]. During the 7-year period analysed here, Niger experienced a serogroup X outbreak in 2006 [3] and the responsible epidemic strain was ST-181 (Table 1). Serogroup X, however, declined later on in Niger and represented less than 1% of the isolates in 2010 [4]. Delrieu and coworkers reported a serogroup X outbreak in one district in Togo in 2007 [35]. Although no isolates had been stored, MLST on cerebrospinal samples revealed ST-181 [35]. This serogroup X strain was present in Burkina Faso in a small fraction (4%) of the cases in 2007, but emerged as an important cause of disease in 2010 where it was identified in 7 of our 10 meningococcal isolates. Serogroup X outbreaks occurring in northern and central regions of Burkina Faso in 2010 have been documented [35]. In 2009, serogroup X ST-181 had been identified in 0.44% of healthy carriers in Burkina Faso [28], with a higher prevalence in the north-east district of Kaya, close to Niger (1.05%). The circulation of serogroup X meningococci among asymptomatic carriers and the high incidence of serogroup X disease before MenAfriVac introduction should be kept in mind when evaluating possible serogroup replacement following use of the vaccine. The possible spread of serogroup X has to be closely monitored in the coming years as there are yet no available vaccines against this serogroup.

Interestingly, serogroup X meningococcal disease also emerged in Kenya and in Uganda in 2006, but these cases were caused by a strain unrelated to the ST-181 complex [8].

### Conclusions

Our data show that before introduction of MenAfriVac in the African meningitis belt, serogroup A *N. meningitidis* of the ST-5



complex remains the predominant disease-causing clonal complex, with two STs (ST-7 and ST-2859) contributing to severe outbreaks in different countries. The situation, however, is heterogeneous both temporally and geographically. After the emergence of serogroup X ST-181 complex in Niger in 2006, the clone became a significant cause of disease in Burkina Faso in 2010 before vaccine introduction. Improved laboratory confirmation of meningitis cases is essential to assess changes in the disease epidemiology. Culture of the disease-causing organism and their genotyping are necessary to a better understanding of the bacterial population dynamics.

## Acknowledgments

This study is based in part on the strain collection assembled by Pierre Nicolas at the French Forces Institute of Tropical Medicine, Le Pharo, Marseille, France. Upon Pierre's retirement in 2010 large parts of Le Pharo's strain collection were transferred to the WHO Collaborating

Centres in Oslo, Atlanta and Paris. We are greatly indebted to Pierre Nicolas and all colleagues who have contributed to that strain collection. We have been unable to get into contact with Dr. Nicolas after his retirement, and thus, we cannot acknowledge by names the contributors to his strain collection. We greatly apologize for this.

The excellent technical assistance of Torill Alvestad, Anne Marie Klem, Jan Oksnes, Jeni Vuong, Raydel Anderson, Stephanie Schwartz and Eva Hong is acknowledged.

This publication made use of the *Neisseria* Multi Locus Sequence Typing website (<http://pubmlst.org/neisseria/>) sited at the University of Oxford.

## Author Contributions

Conceived and designed the experiments: DAC LWM MKT. Performed the experiments: RO DK FB SS LB. Analyzed the data: PAK XW. Contributed reagents/materials/analysis tools: RO DK FB SS LB. Wrote the paper: DAC PAK XW.

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### **Errata (rectified typographic errors)**

- Page 46, chapter 4.2.2, first sentence, second line: “in October-November 2012 (S5)” rectified to “in October-November 2010 (S5)”
- Page 57, table 2, third column (“ELISA (Total IgG)”), 6<sup>th</sup> row (“Control”): “Negative control: purified IgG” rectified to “Negative control: purified IgM”
- Page 64, chapter 5.7, 4<sup>th</sup> paragraph, last sentence: “mean, 255 ng/ml” rectified to “geometric mean, 255 ng/ml”
- Page 70, chapter 6.2.1, last paragraph: delete “and” at the end of this sentence: “In 2010, the dominating serogroup was NmX and the prevalence peaked in 5-9-year-olds and.”
- Page 80, chapter 7.1, first paragraph, second line: “ST2859” rectified to “ST-2859”